



3º CONGRESO
LATINOAMERICANO DE
HEMATOPATOLOGÍA
SÃO PAULO | 2023



Sociedade
Brasileira de
PATOLOGIA

DIFFUSE LARGE CELL LYMPHOMAS AND HIGH-GRADE B CELL LYMPHOMAS: MOLECULAR ASPECT

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APOYO



Diffuse Large B-cell Lymphomas

A Heterogeneous group of diseases

Diffuse large B-cell lymphoma, not otherwise specified (NOS)

Germinal center B-cell subtype

Activated B-cell subtype

DLBCL, topographic site/ microenvironment: CNS and Testicular lymphoma, Primary mediastinal LBCL, T-cell/Histiocytic rich Large B Cell Lymphoma,

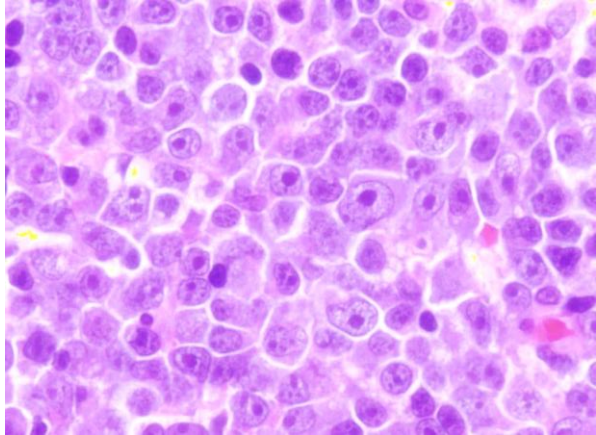
LBCL, related to viral infection (EBV, HHV8): EBV+ DLBCL, Primary Effusion Lymphoma

LBCL Terminal B-cell differentiation : Plasmablastic lymphoma, ALK+ LBCL

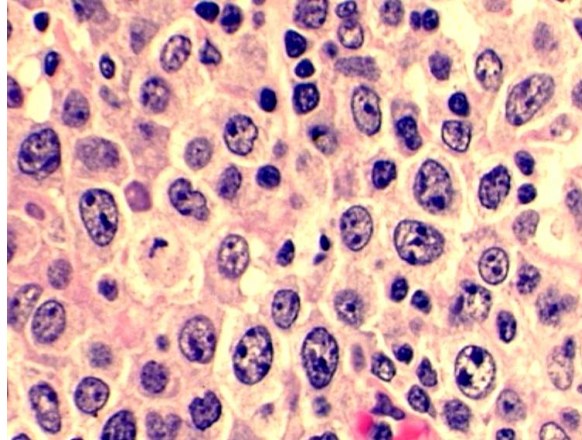
High grade B-cell lymphomas: HGBCL MYC and BCL2 rearrangements, HGBCL MYC and BCL6-R; HGBCL, NOS

DLBCL, NOS: Heterogeneous morphology and phenotype

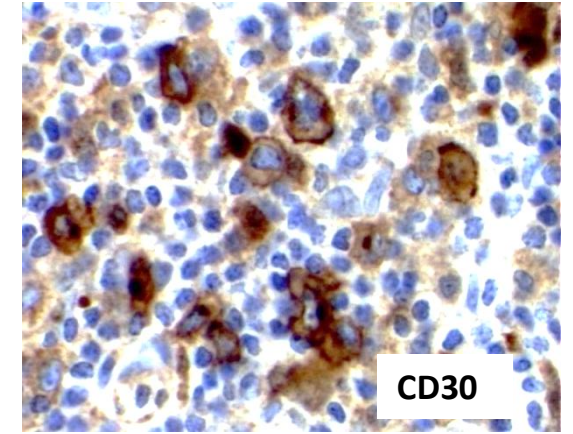
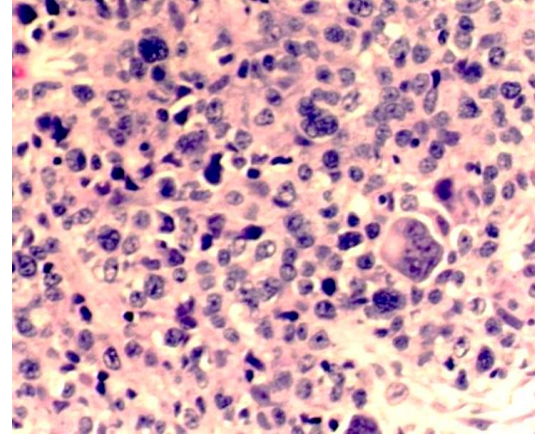
Immunoblastic



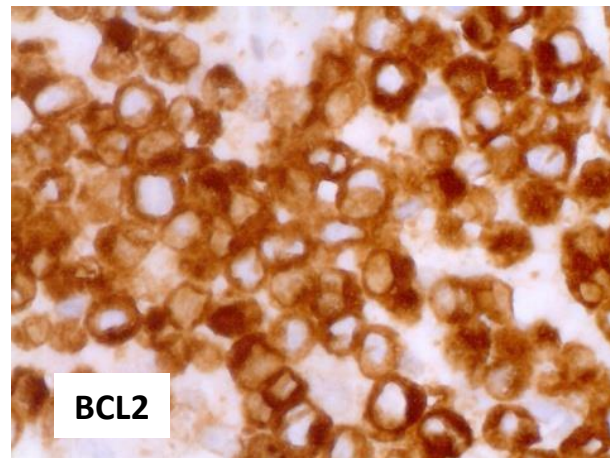
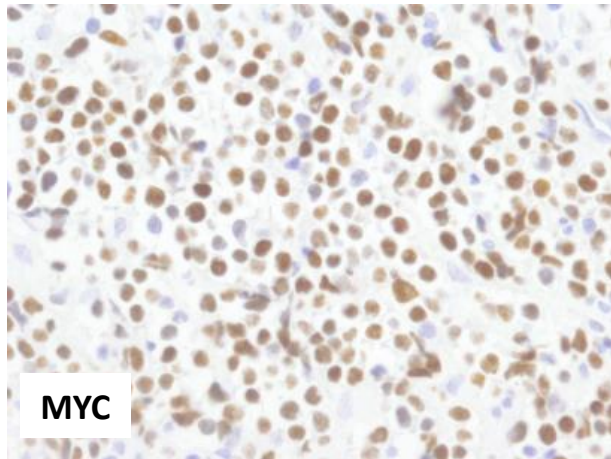
Centroblastic



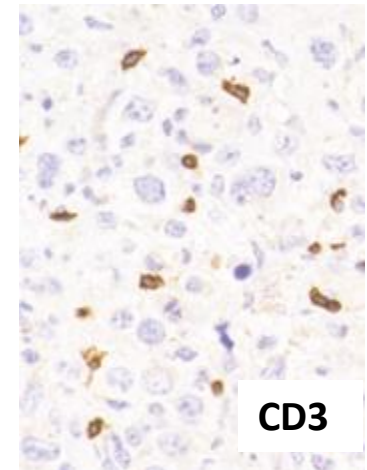
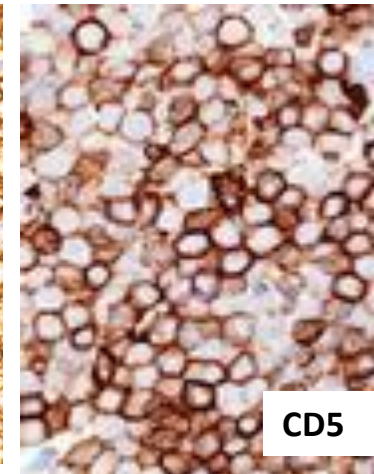
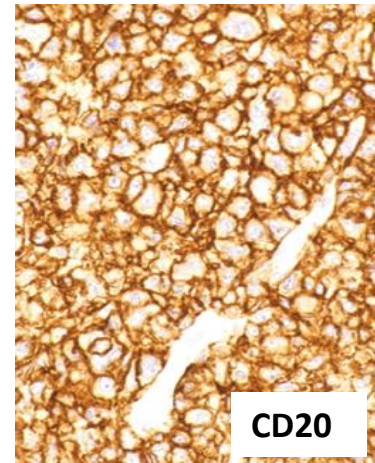
Anaplastic



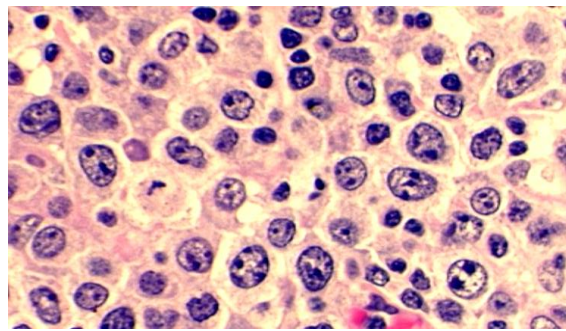
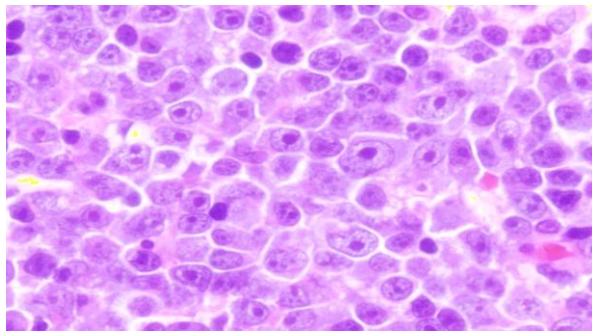
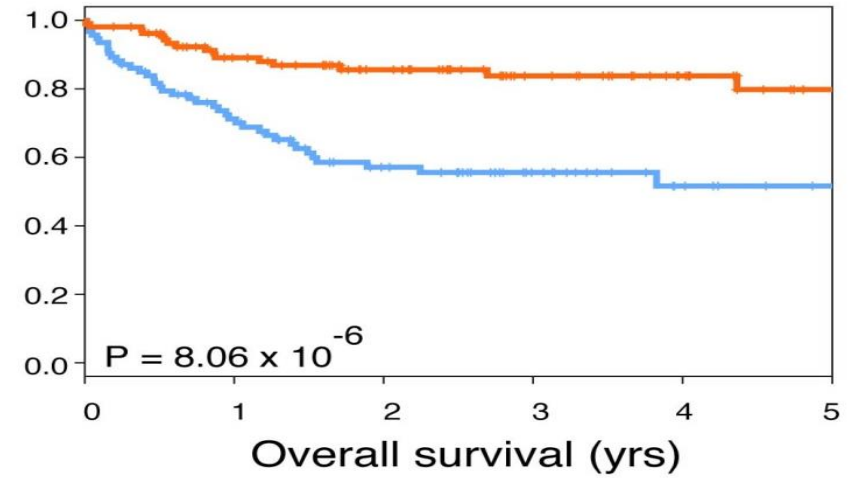
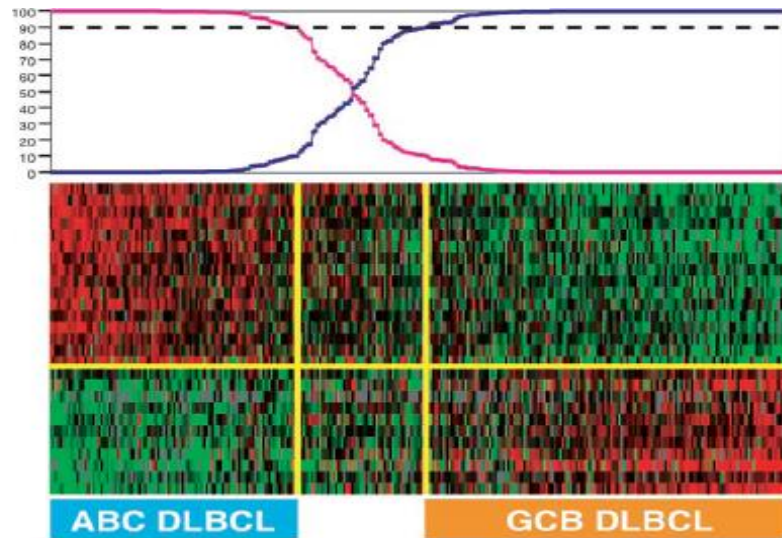
Double Expressors



CD5 + DLBCL



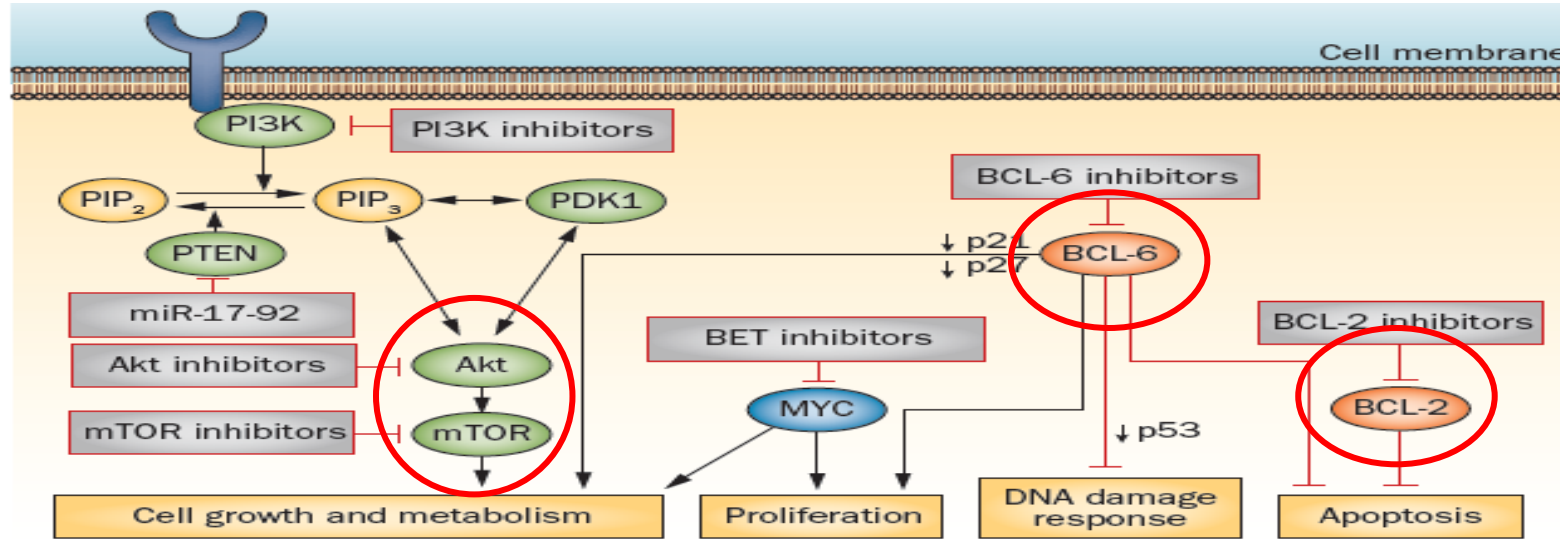
Gene Expression Profiling Identifies two Molecular Subtypes of DLBCL



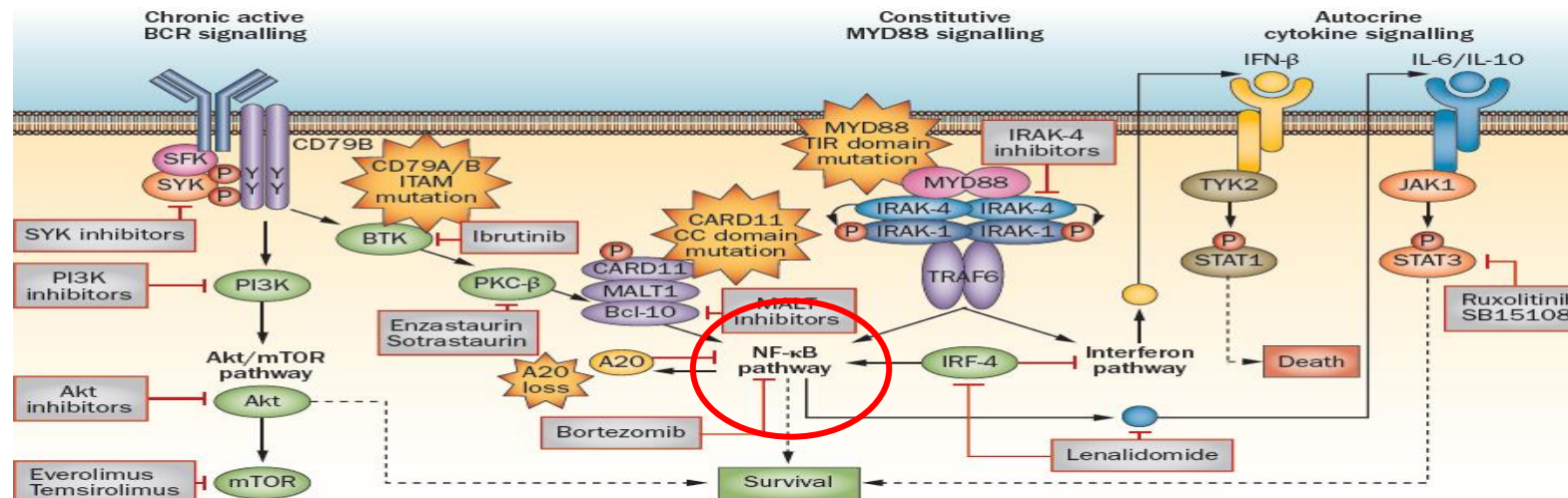
■ GCB DLBCL
■ ABC DLBCL

Molecular Subtypes of DLBCL Have Different Molecular Pathogenesis

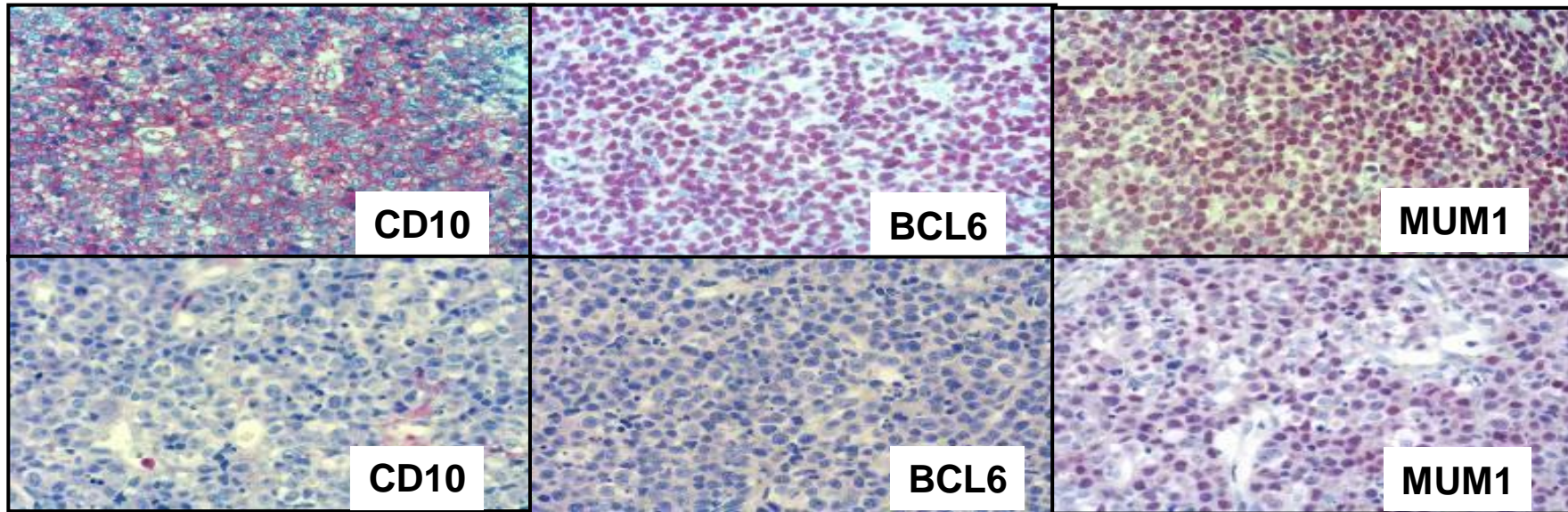
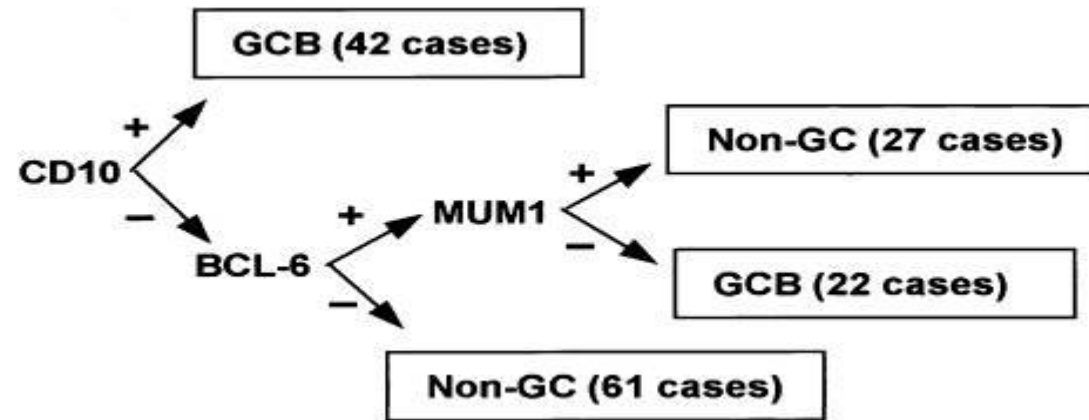
GCB



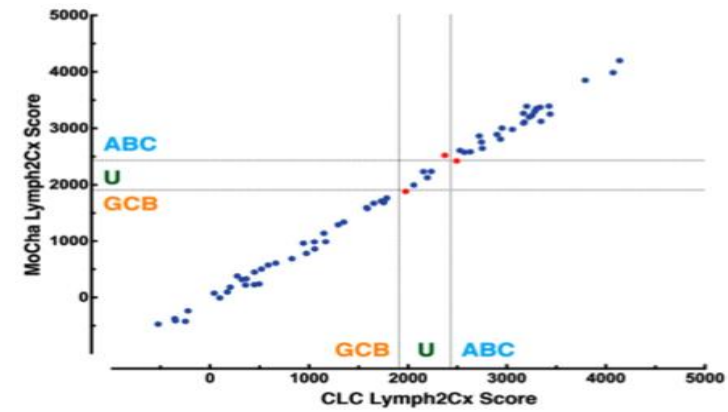
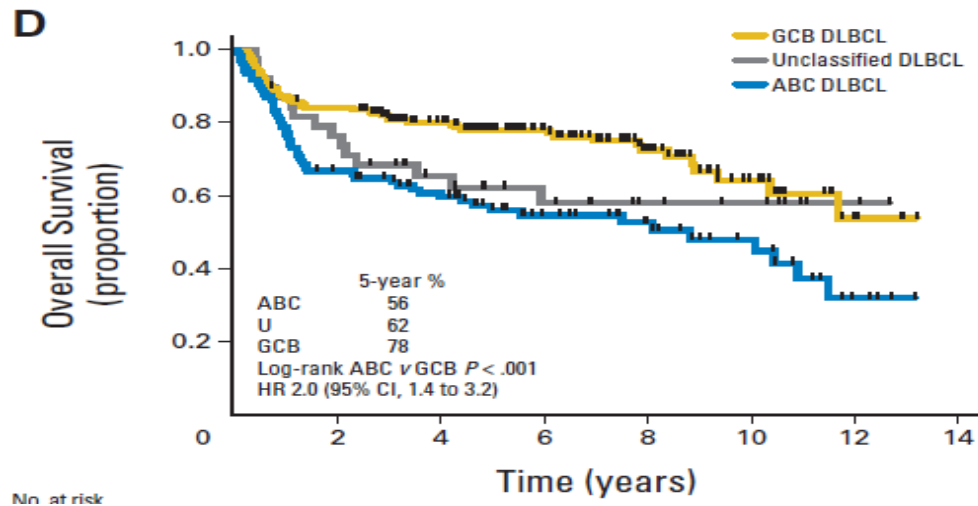
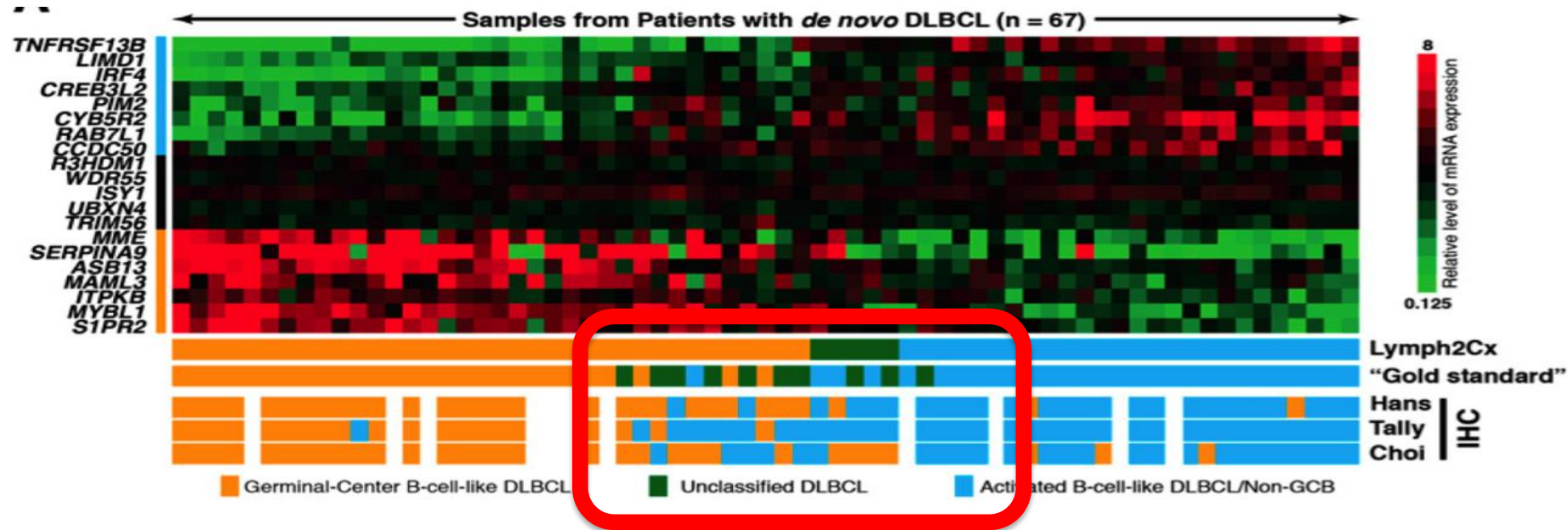
ABC



DLBCL Subgroups Have Different Phenotype

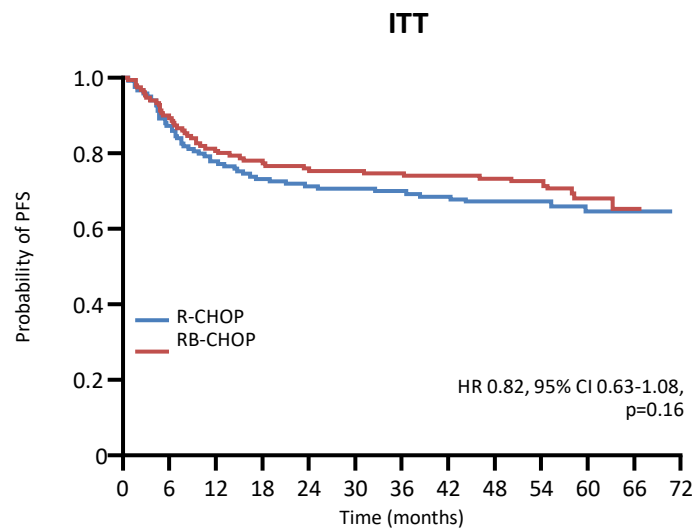


RNA based Assay for molecular classification of DLBCL

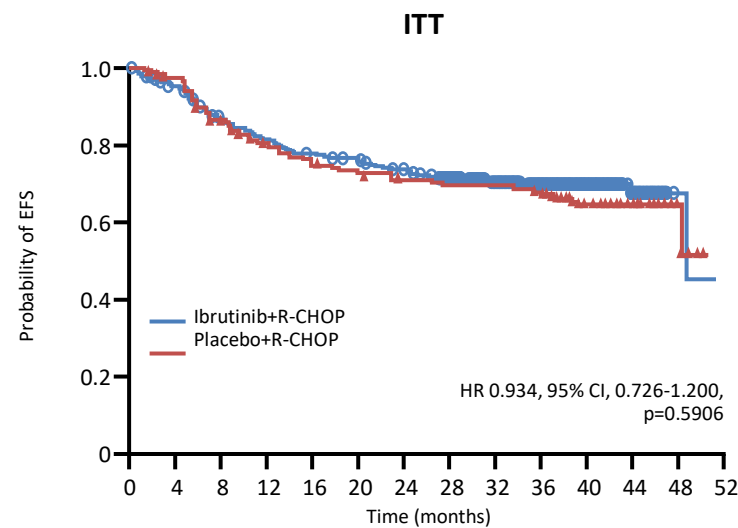


No survival benefit with bortezomib, ibrutinib or lenalidomide plus R-CHOP versus R-CHOP in DLBCL

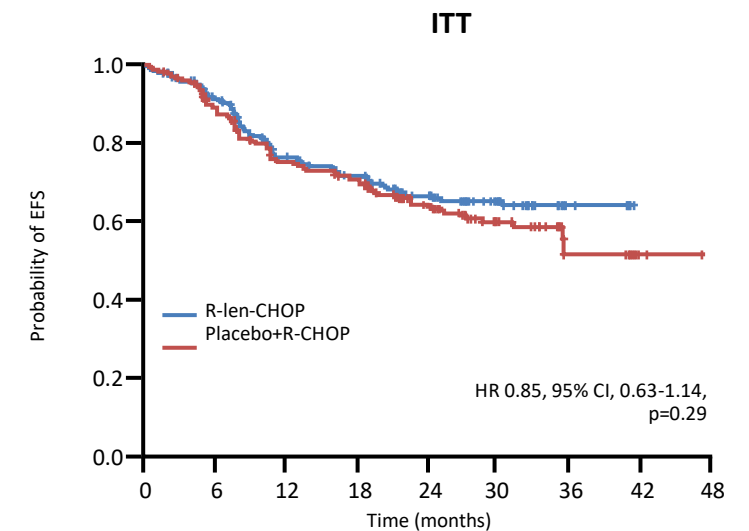
No significant difference in PFS between **bortezomib-R-CHOP** and R-CHOP¹



No significant difference in EFS between **ibrutinib-R-CHOP** versus R-CHOP²



No significant difference in EFS between **lenalidomide-R-CHOP** versus R-CHOP³ (ABC-DLBCL)



1. Davies A. et al. Lancet Oncol 2019;20:649–62
2. Younes A. et al. J Clin Oncol 2019;37:1285–95
3. Vitolo U. et al. Hematol Oncol 2019;37:36–7

These data are from different studies with different study designs; they are not intended to be directly compared.
 CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone;
 EFS, event-free survival; ITT, intent-to-treat; len, lenalidomide; R, rituximab

Molecular Genetic Subtypes of DLBCL



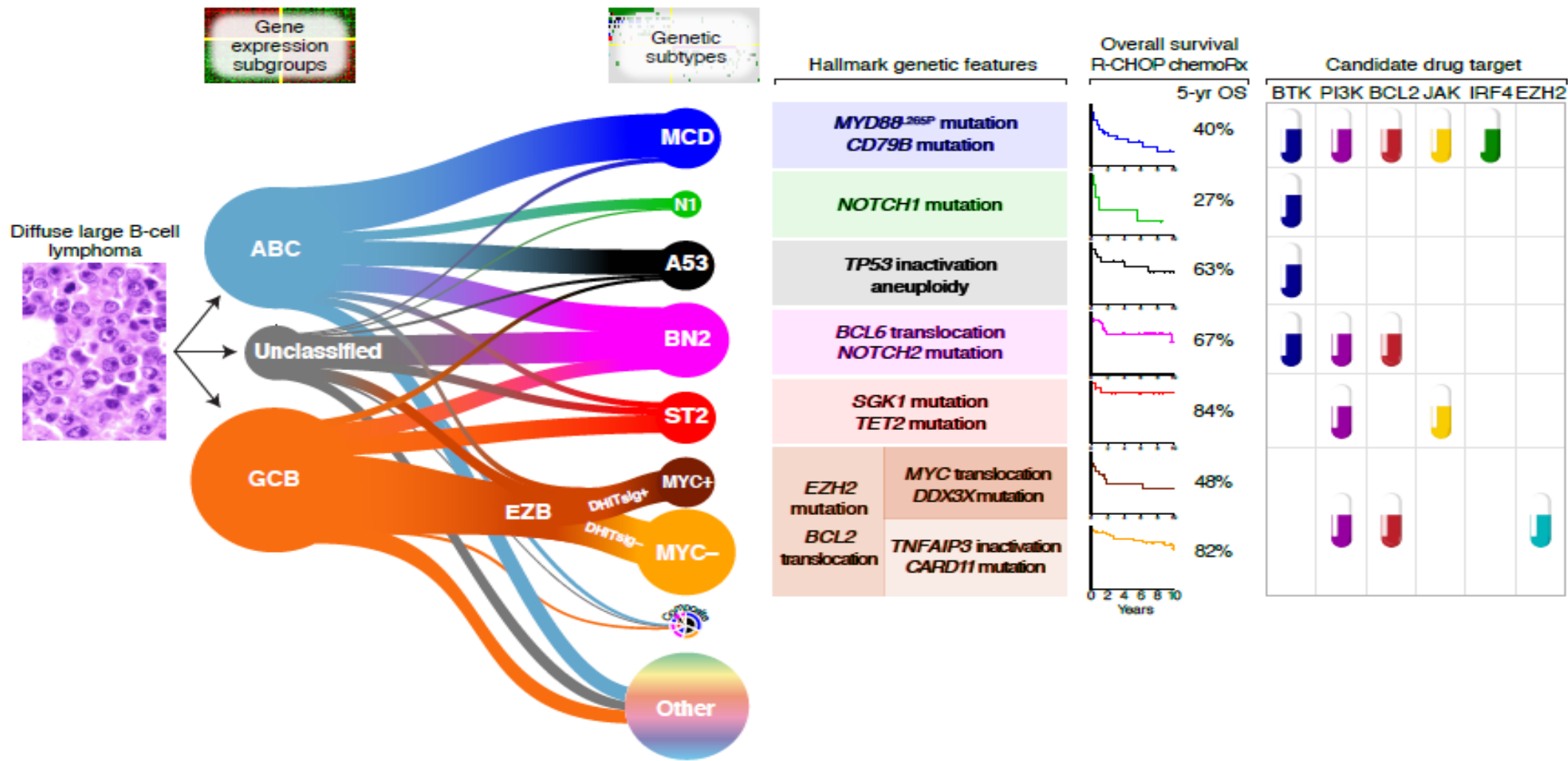
Wright 2020	Chapuy 2018	Lacy 2020	Hallmark drivers	%
MCD	C5	MYD88	MYD88/CD79B	14-21
BN2	C1	NOTCH2	tBCL6/ NOTH2	16-19
EZB-MYC-	C3	BCL2	EZH2 tBCL2	13-18
EZB-MYC+			EZH2/MYC	
A53	C2		TP53 Aneuploidy	7-21
ST2	C4	SOCS1/TET /SGK1	SOCS1/TET/ SGK1	5-17
N1		NEC	NOTCH1	3
UNCLASS				37



- Genetic subgroups capture biological complexity but not ready for clinical use
- Expectation of transitioning to a molecular genetic classification in the near future

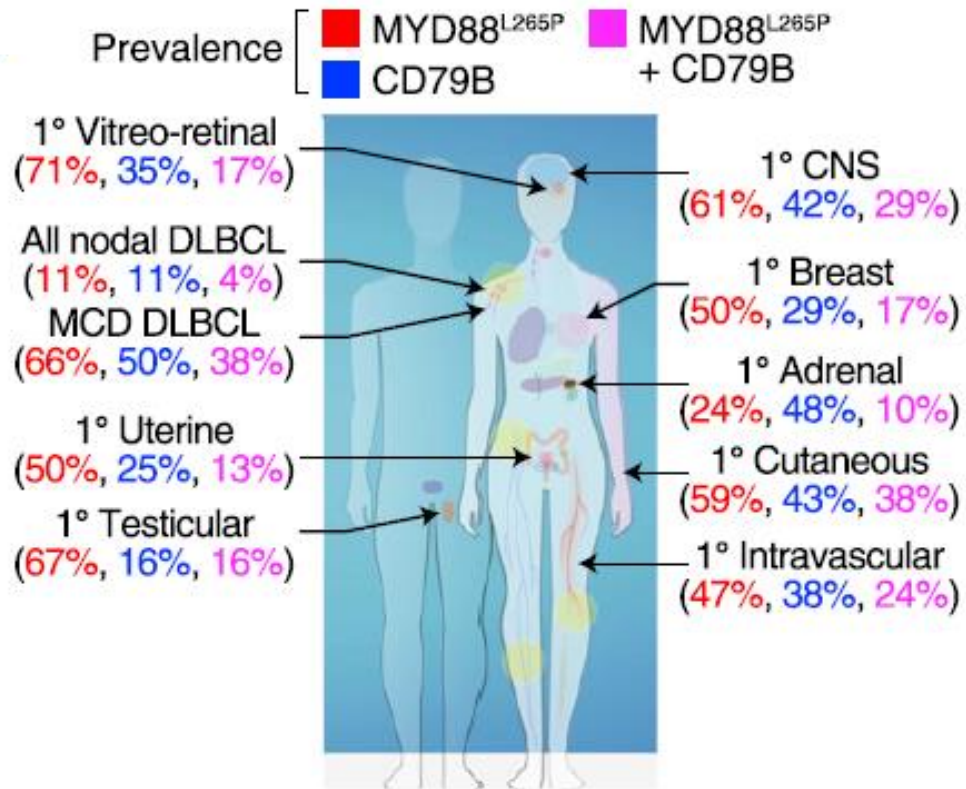
Wright et al Cancer Cell 2020; Chapuy et al Nat Med 2018; Lacy et al Blood 2020

DLBCL Genetic Subgroups Based on Mutational Profile



Wright G et al Cancer Cell 2020 ; De Leval et al Blood 2022,

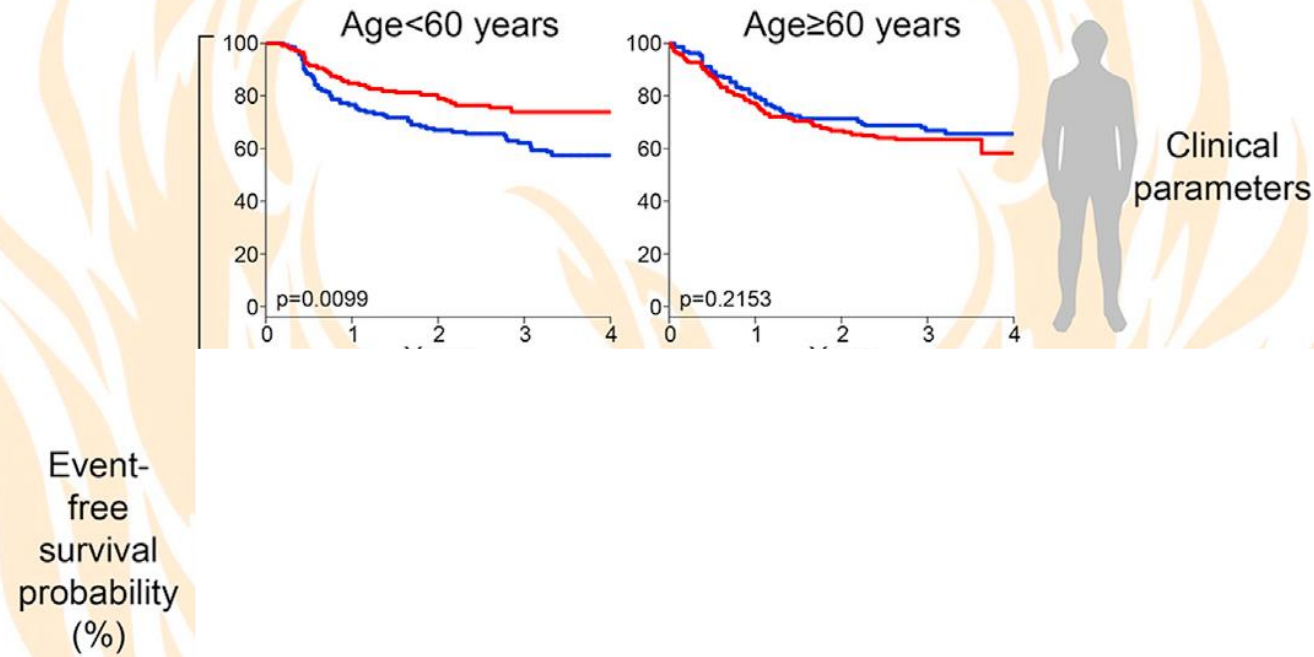
Extranodal DLBCL ABC (Non-GCB)



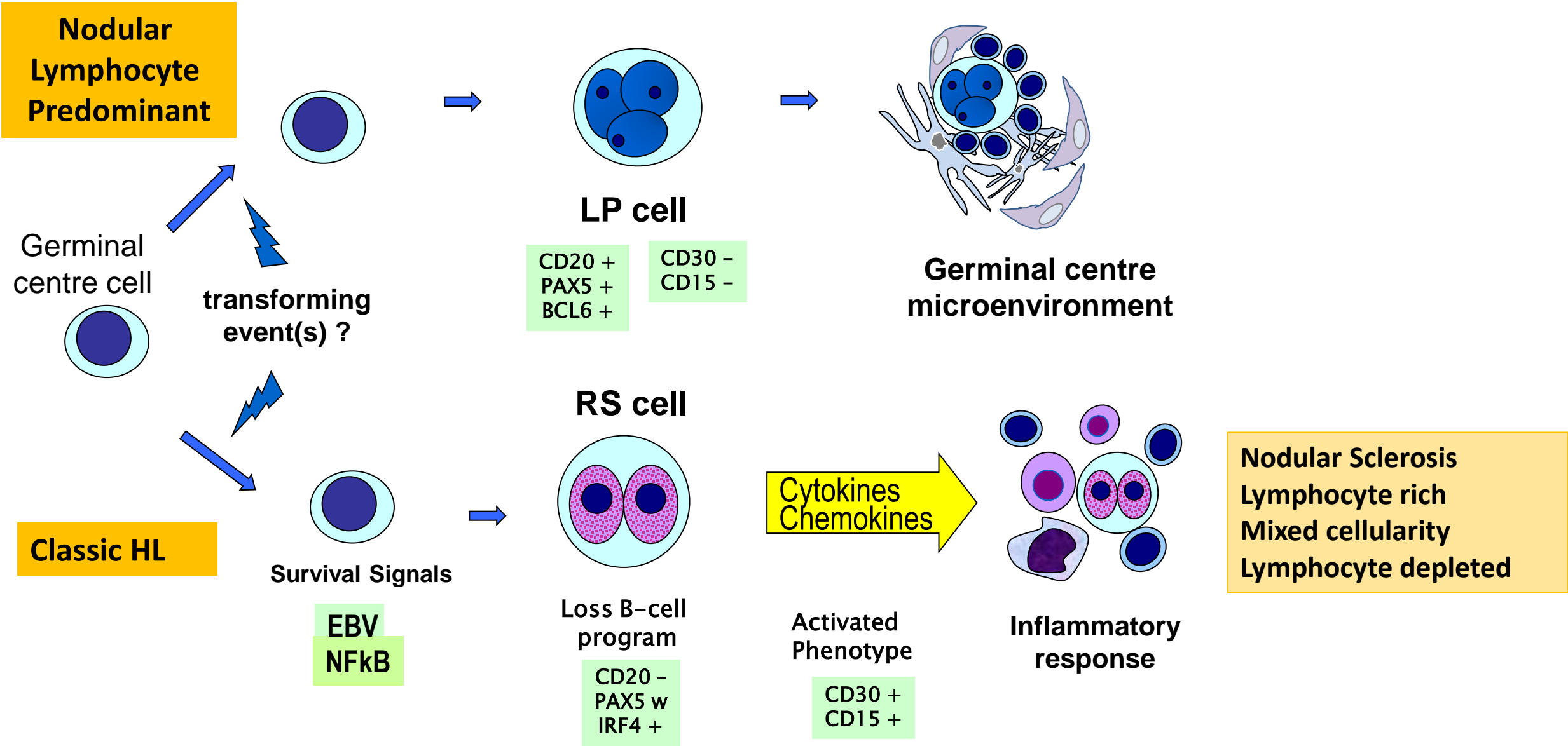
- Extranodal DLBCL, ABC, share biological features (MYD88/CD79B)
- Close relationship of primary CNS and testicular DLBCL
 - Genetic alterations in genes involved in immune escape (HLA, CD274/PDL1LG2)
 - Relapses of PCNS in testes, breast and peripheral nervous system.
 - Relapses of PTL in brain (*BCL6* and/or PDL rearrangements)
- Some subtypes better defined by the topographic site (IVLBCL)
- Not enough information on the relationship between tumors in different extranodal sites (e.g. Breast, Adrenal, Uterine)

Phoenix Phase III Clinical Trial in Previously Untreated Non-GCB Diffuse Large B Cell Lymphoma

■ Ibrutinib + R-CHOP
■ Placebo + R-CHOP



Hodgkin Lymphoma: Two Diseases



Nodular Lymphocyte Predominant

Germinal centre cell

transforming event(s) ?

Classic HL

Survival Signals

EBV
NFkB

LP cell

CD20 +
PAX5 +
BCL6 +
CD30 -
CD15 -

Germinal centre microenvironment

RS cell

Loss B-cell program

CD20 -
PAX5 w
IRF4 +

Cytokines
Chemokines

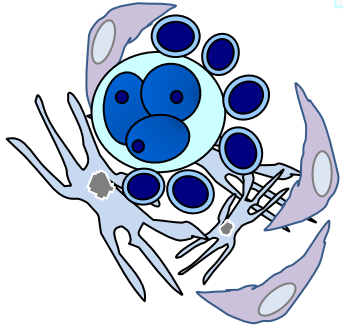
Activated Phenotype

CD30 +
CD15 +

Inflammatory response

Nodular Sclerosis
Lymphocyte rich
Mixed cellularity
Lymphocyte depleted

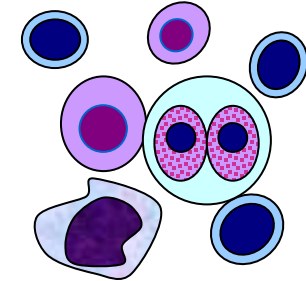
ICC Proposal in Hodgkin Lymphoma



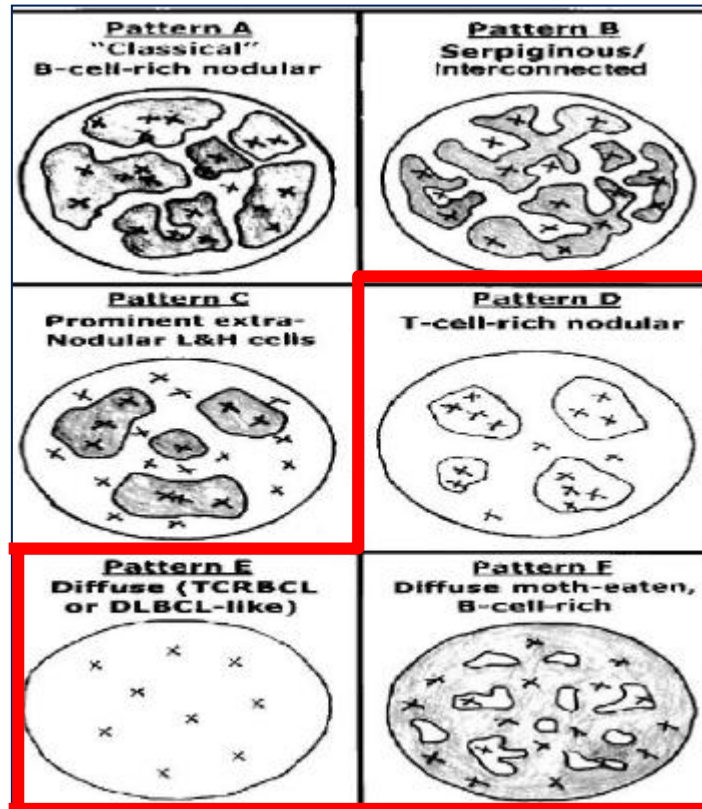
~~Nodular lymphocyte predominant Hodgkin Lymphoma~~

Nodular Lymphocyte Predominant B-cell lymphoma
Related to THRLBCL – a continuum

Classical Hodgkin Lymphoma



Clinical management
distinct from CHL



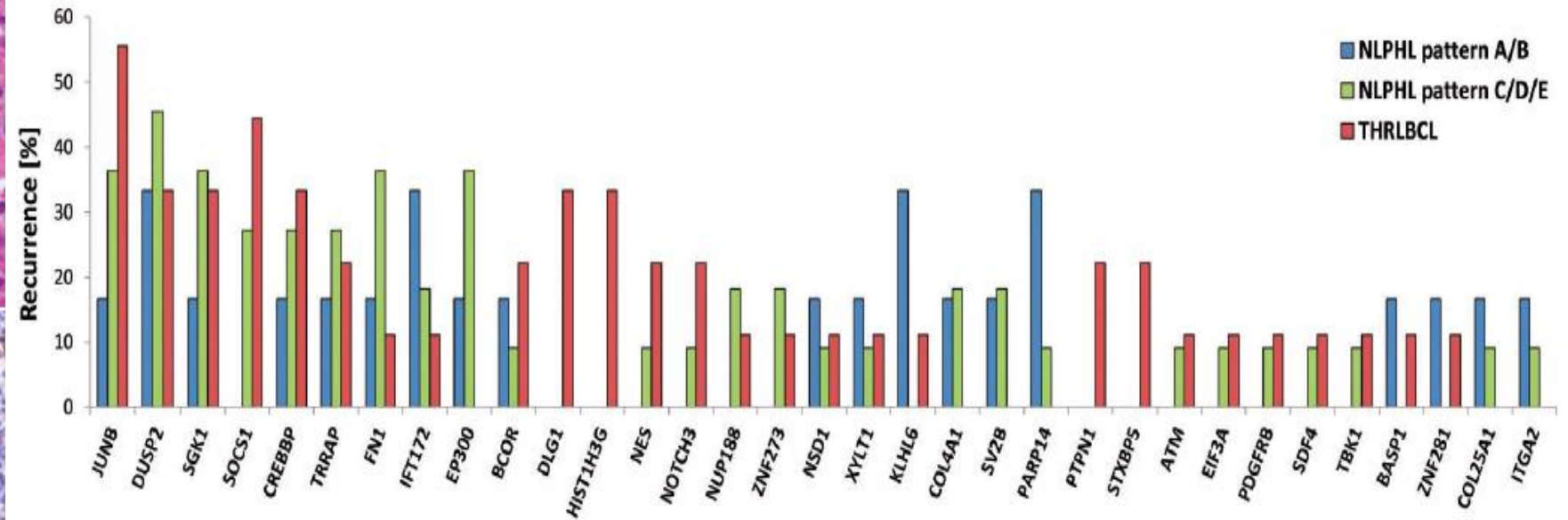
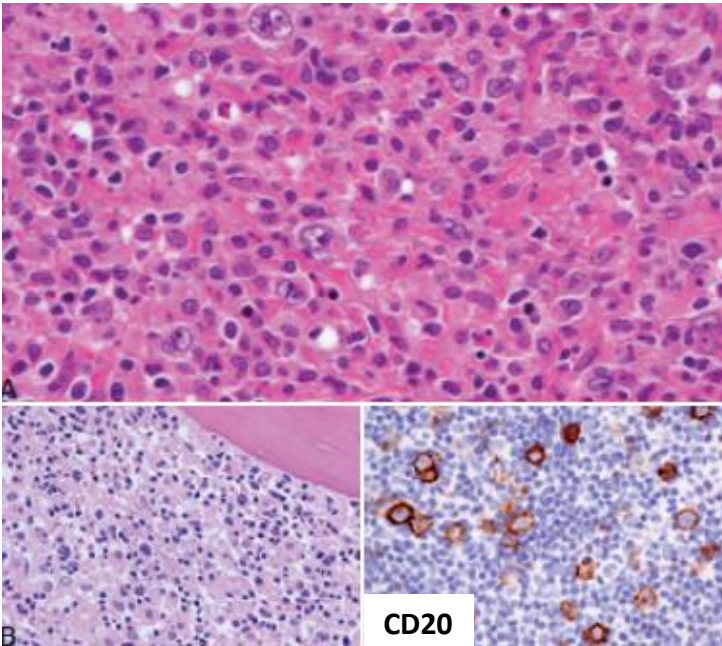
Grade 1 Classical
patterns



Grade 2
Variant patterns

Advanced disease
Higher relapse rate
For Grade 2
(variant patterns)

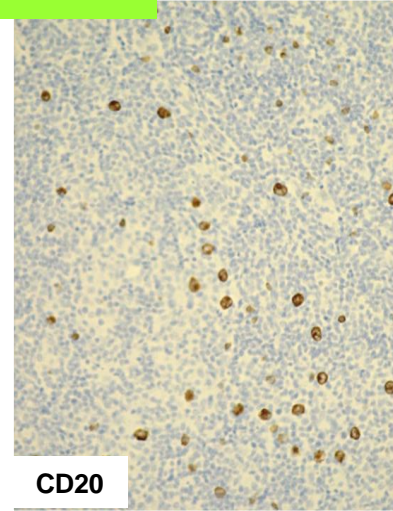
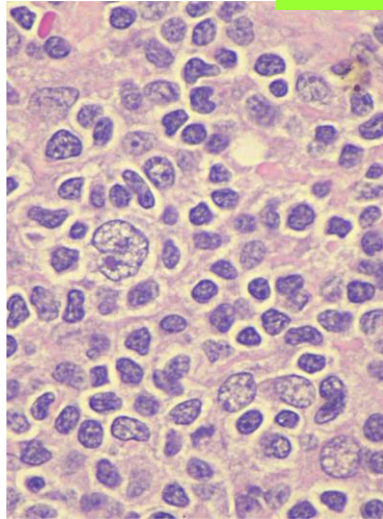
Mutational profile in T/Histiocyte Rich LBCL and NLPLBCL



***JUNB, DUSP2, SGK1, SOCS1 and CREBBP* are frequently mutated in THRLBCL and NLPLBCL**

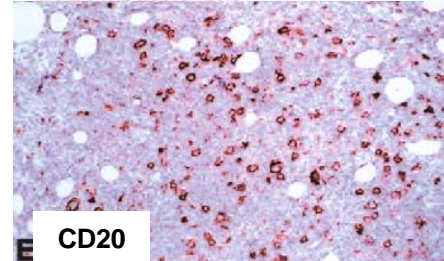
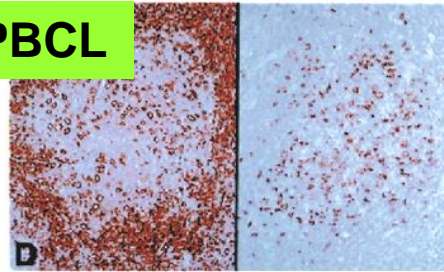
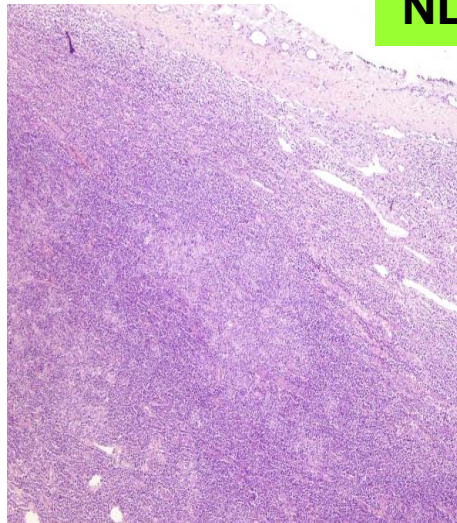
Relationship between TH-cell Rich LBCL and NLPBCL

THRLBCL



CD20

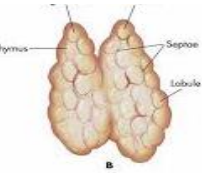
NLPBCL



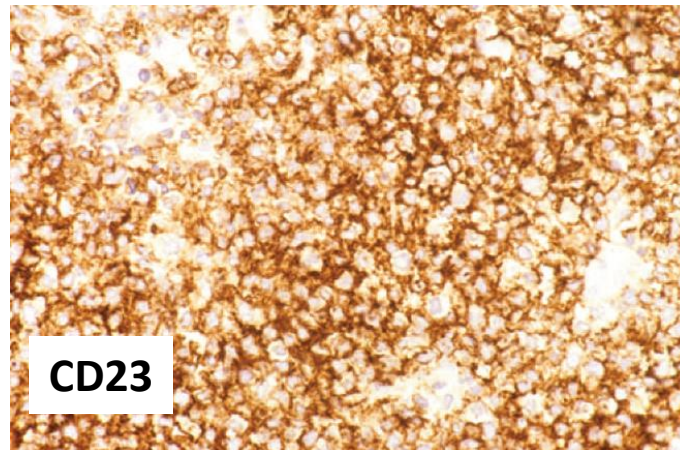
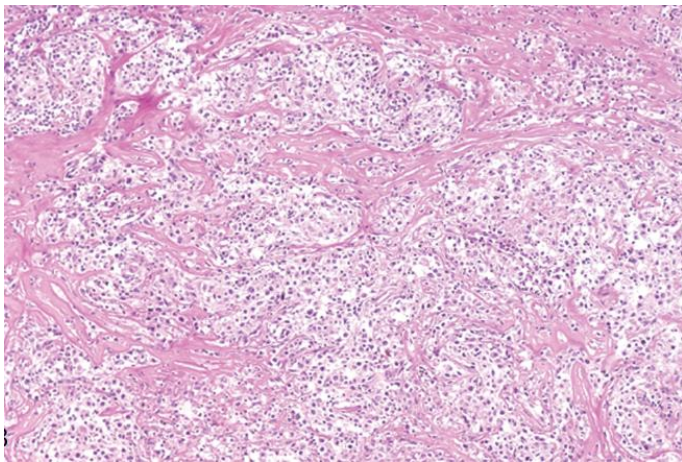
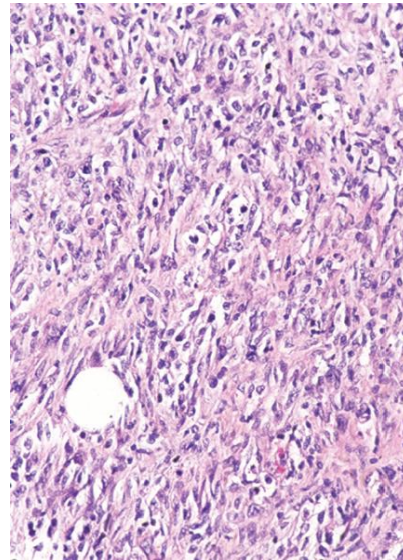
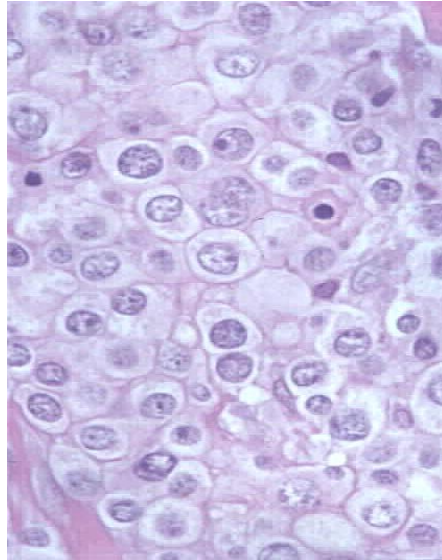
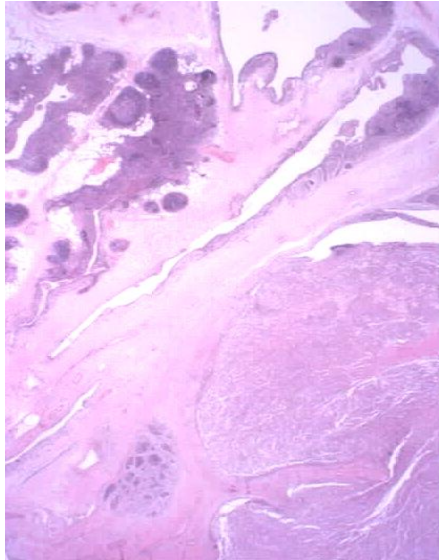
CD20

- NLPBCL can show a THRLBCL-like areas
- Tumor cells in NLPBCL and THRLBCL have similar GEP
- Gains of 2p16.1 and losses of 2p11.2 and 9p11.2 recurring aberrations in THRLBCL and NLPBCL
- More complex karyotypes in THRLBCL than in NLPBCL

THRLBCL transformation of NLPBCL



Primary Mediastinal (Thymic) Large B-cell Lymphoma



• Clinical Characteristics

- Young female
- Bulky mediastinal mass
- Frequent extrathoracic relapses

• Phenotype

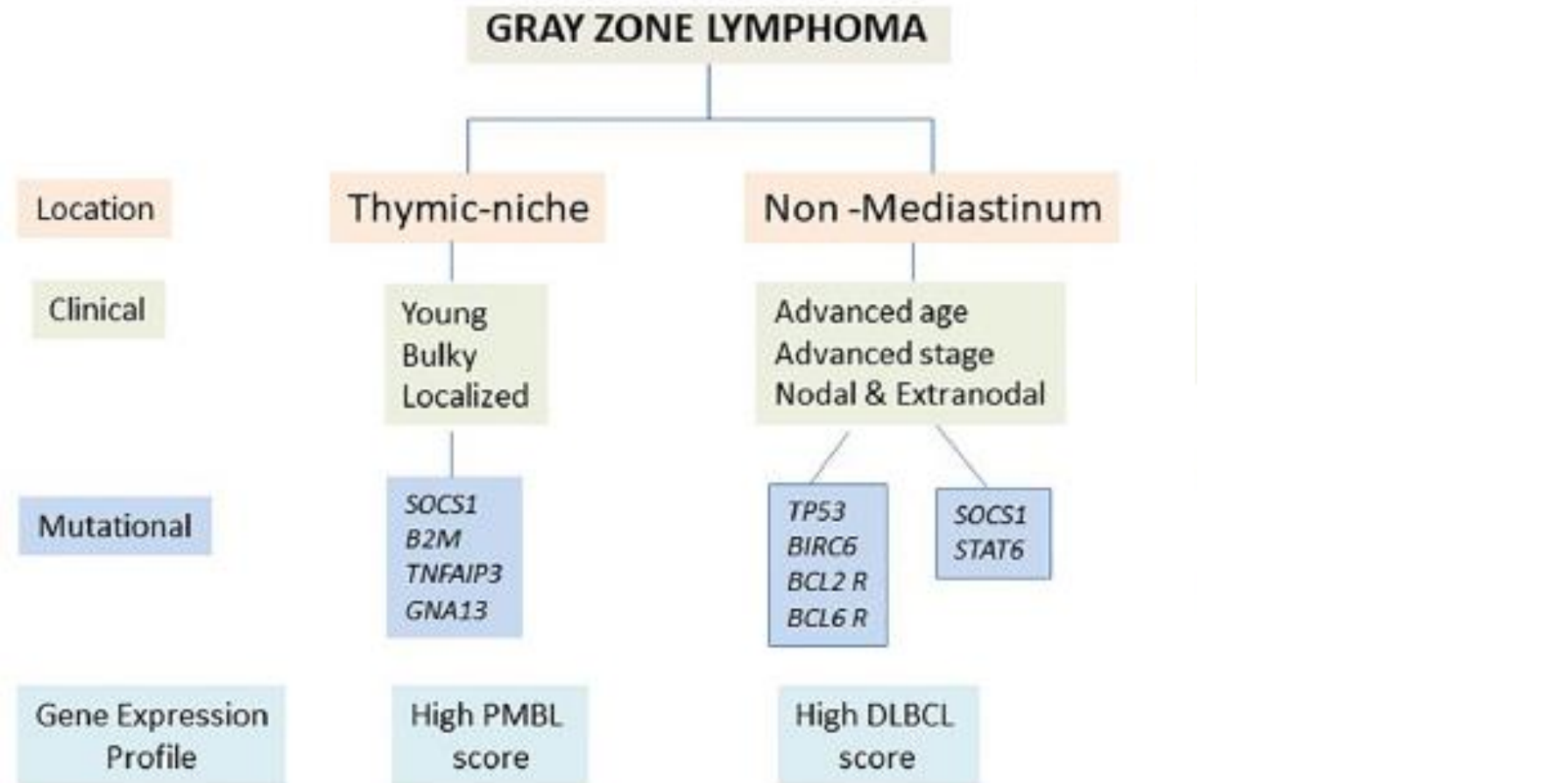
- B-cell markers, Discordant CD79+ Ig-
- CD30+, CD23 +, IRF4+, CD10-

• Molecular Genetic Alterations

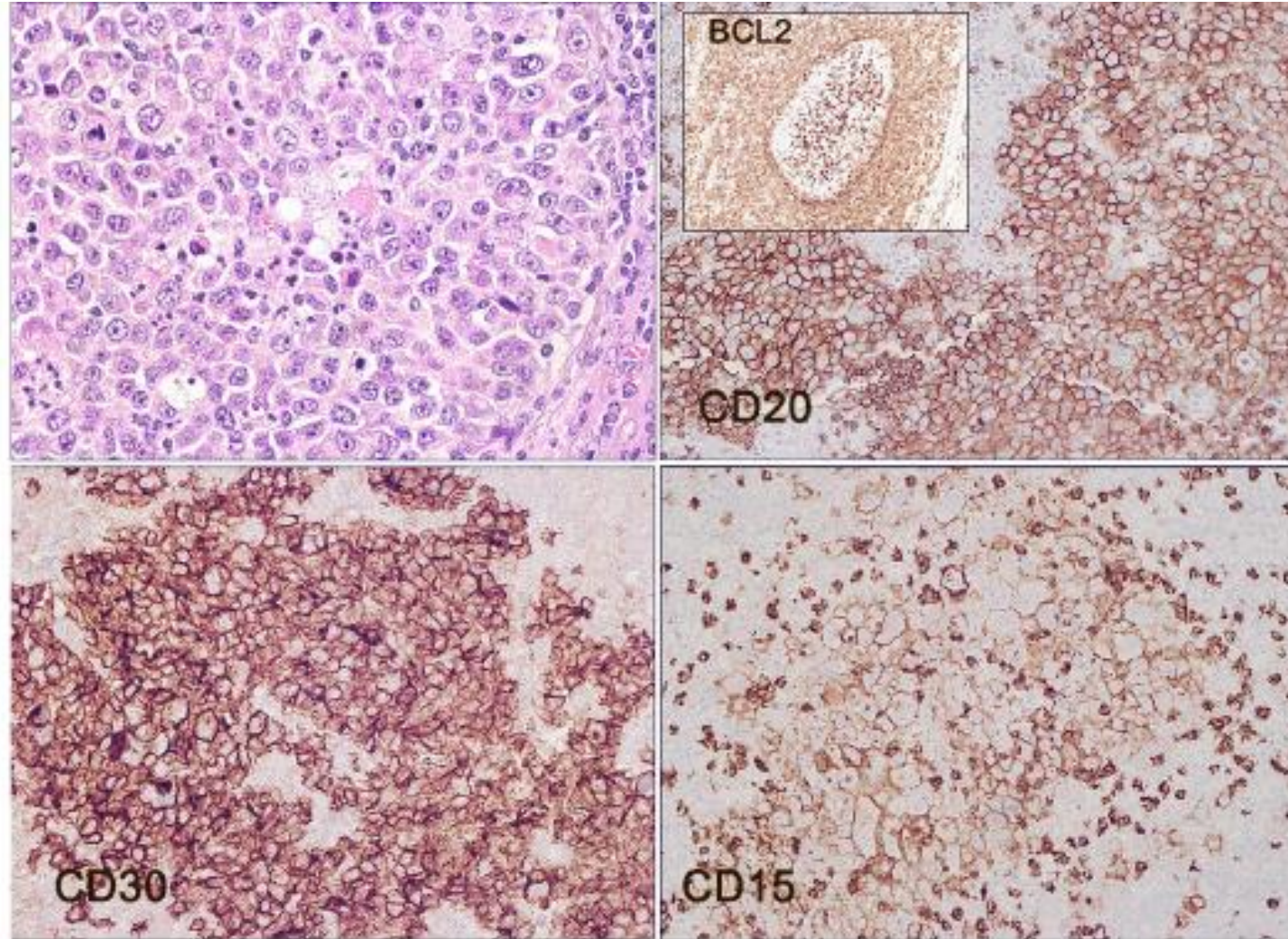
- Immunoscape (CIITA inactivation, CD58)
- JAK/STAT pathway activation (SOCS/STAT6)
- NFkB activation (REL, A20, NFkBIE)

Mediastinal Gray Zone Lymphoma

GZL in extra-mediastinal sites is a different entity related to DLBCL

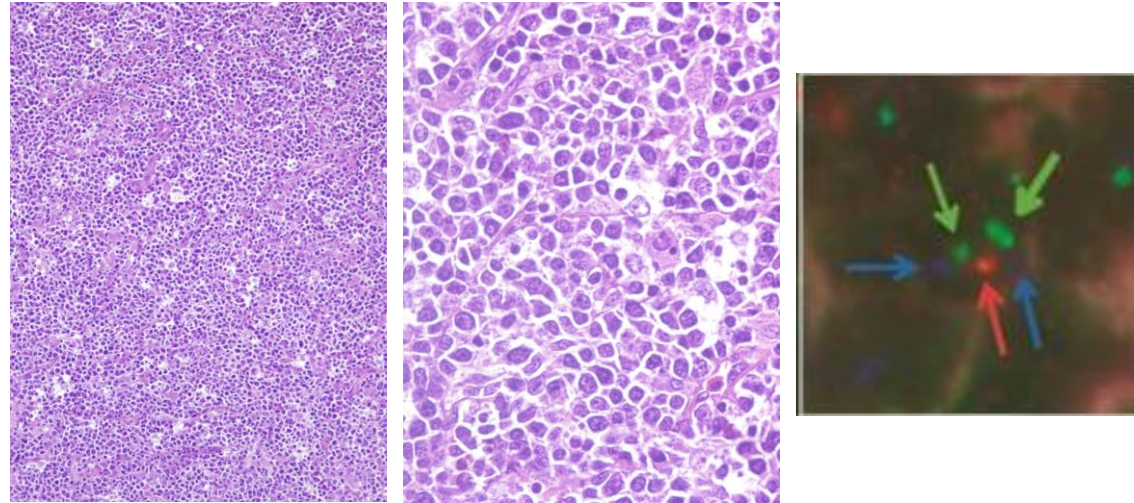


GZL between CHL and DLBCL in extra-mediastinal site is a different entity than mediastinal GZL

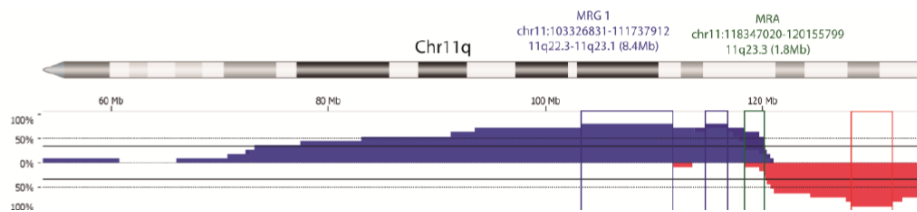


Large B-cell lymphoma with 11q aberration

High-grade lymphoma with 11q aberration (Burkitt-like lymphoma with 11q aberration)



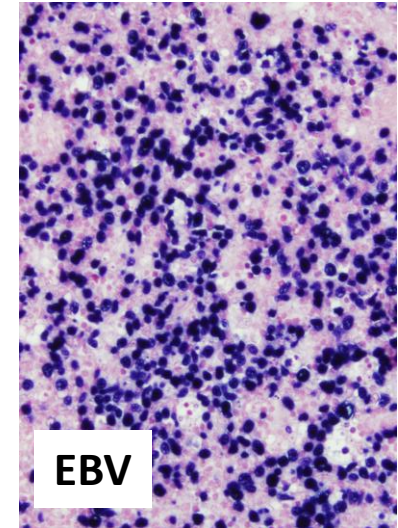
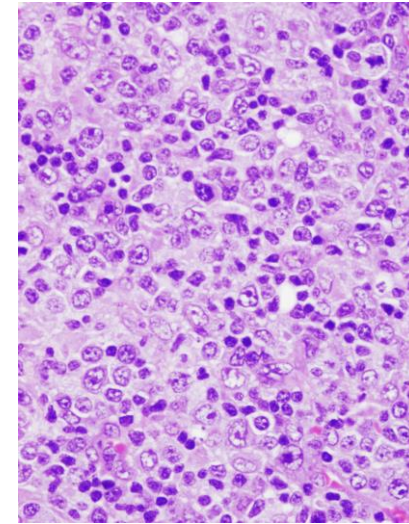
- Children and young adults
- Predominantly nodal
- Spectrum of morphology from Burkitt-like to large cell
- Negative *MYC* rearrangement
- 11q22-q24 gain /11q24-qter loss
- Mutational profile closer to DLBCL
 - No *ID3*, *TCF3*
 - *BTG2*, *GNA13*, *CREBBP*
- Favorable prognosis with current treatment



Clinically Aggressive EBV+ B-cell Neoplasms

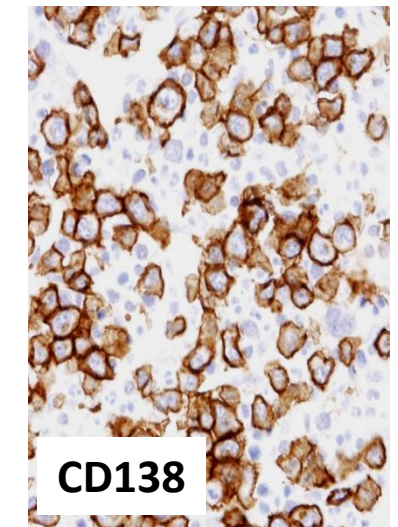
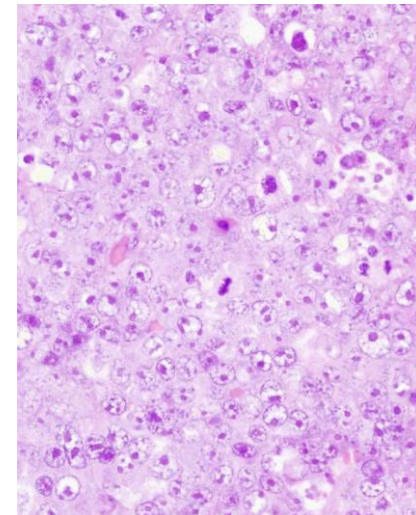
- **EBV-positive Diffuse large B-cell lymphoma, NOS**

- Wide age range, more common in elderly
- Nodal or extranodal
- May have polymorphic background with HRS-like cells
- Non-GCB phenotype; EBV Latency II/ III
- Genomic alterations in NFkB, IL6, JAK/STAT pathways

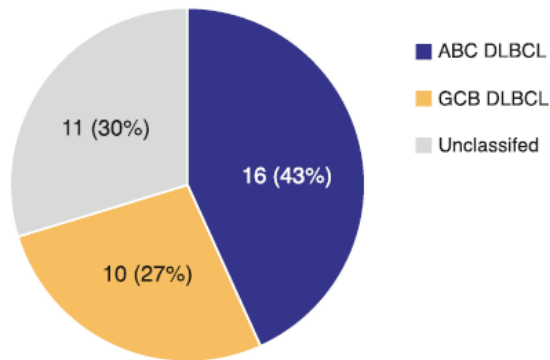


- **Plasmablastic lymphoma**

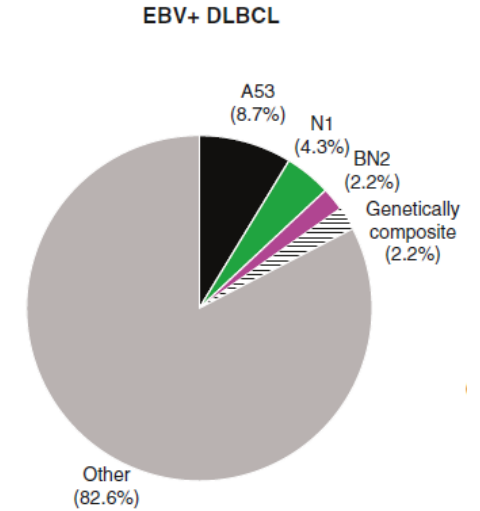
- Congenital or acquired immune deficiency
- Extranodal > Nodal
- Negative mature B cell markers but positive plasma cell
- EBV Latency I
- *MYC* rearrangement
- Secondary PBL (multiple myeloma, CLL, FL) not associated with immune deficiency



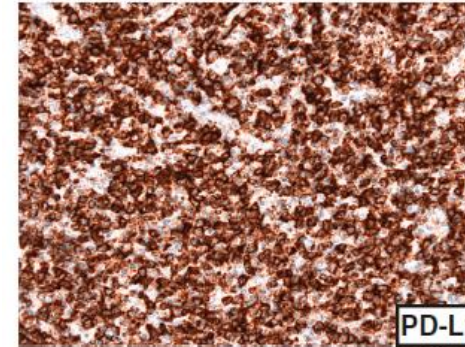
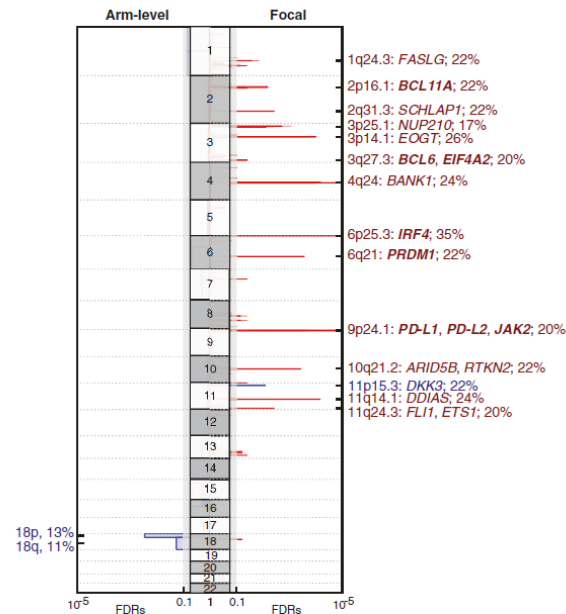
Molecular and Mutational Profile of EBV+ DLBCL



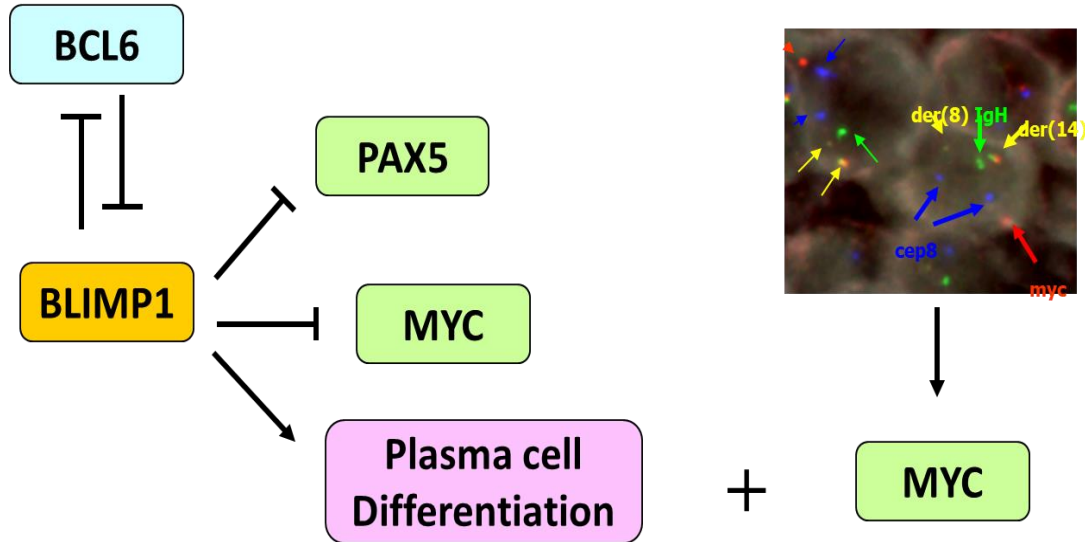
- Chromatin regulators (52%)
- JAK/ STAT alterations (30%)
- NOTCH1/2 22%
- Immune scape 11%
- TP53 7% mutations
- NFKB 7%



Amplifications/ Deletions



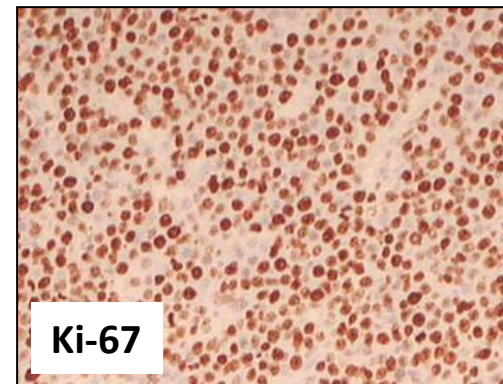
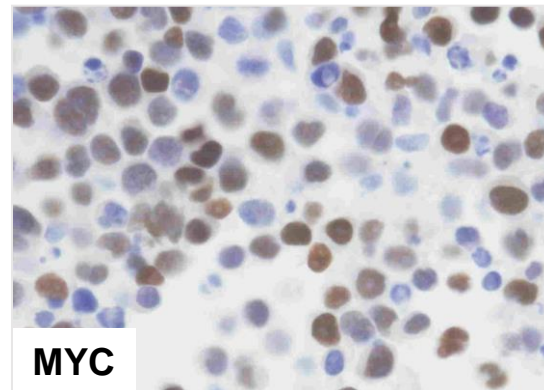
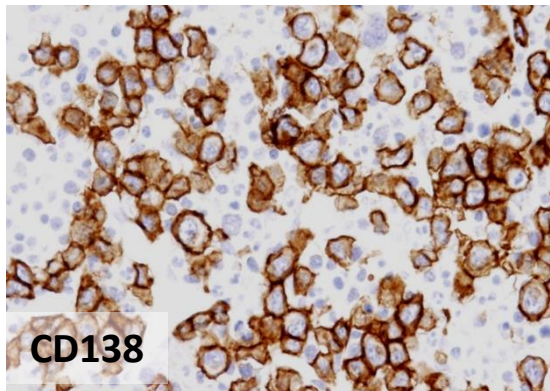
MYC alterations in PBL



40-50% MYC Translocations

- 83 % cases t(8;14) MYC/IgH
- 11% t(8;22) or t(2;22)
- 6% non – IG

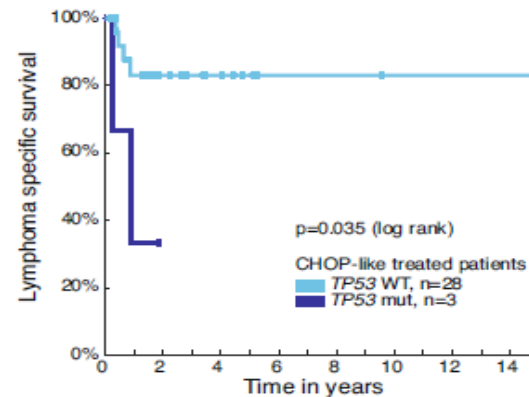
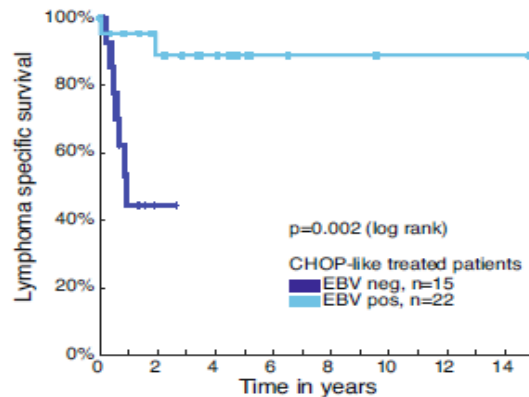
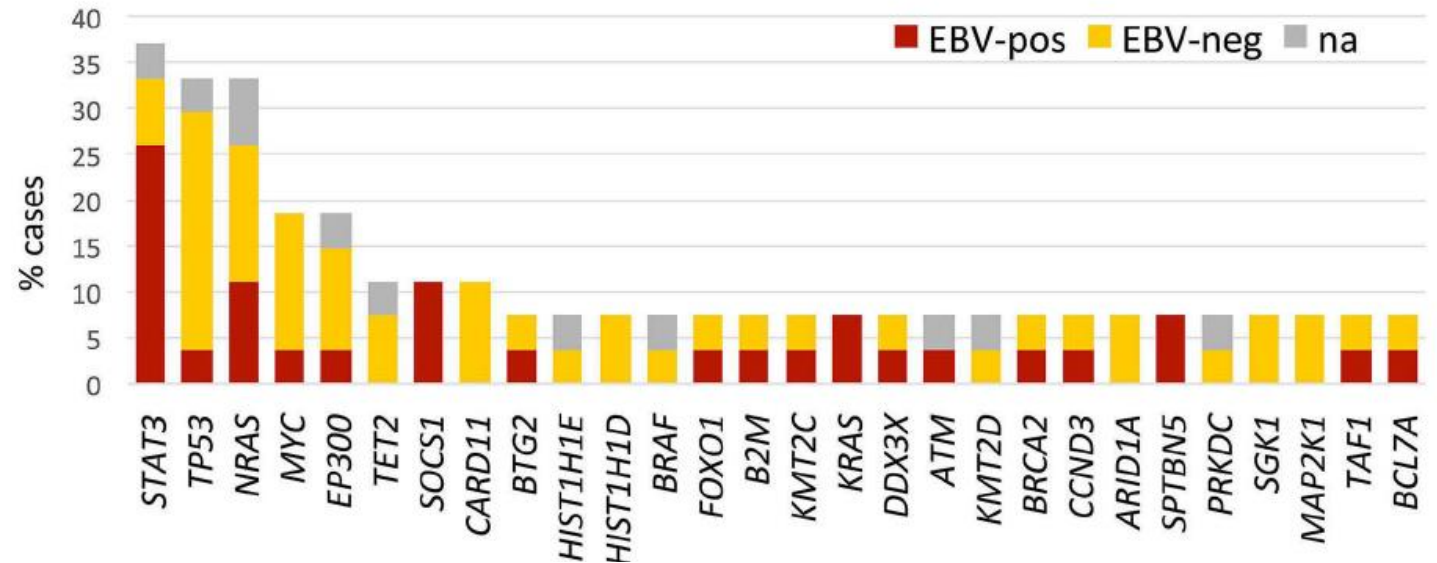
64% MYC protein overexpression



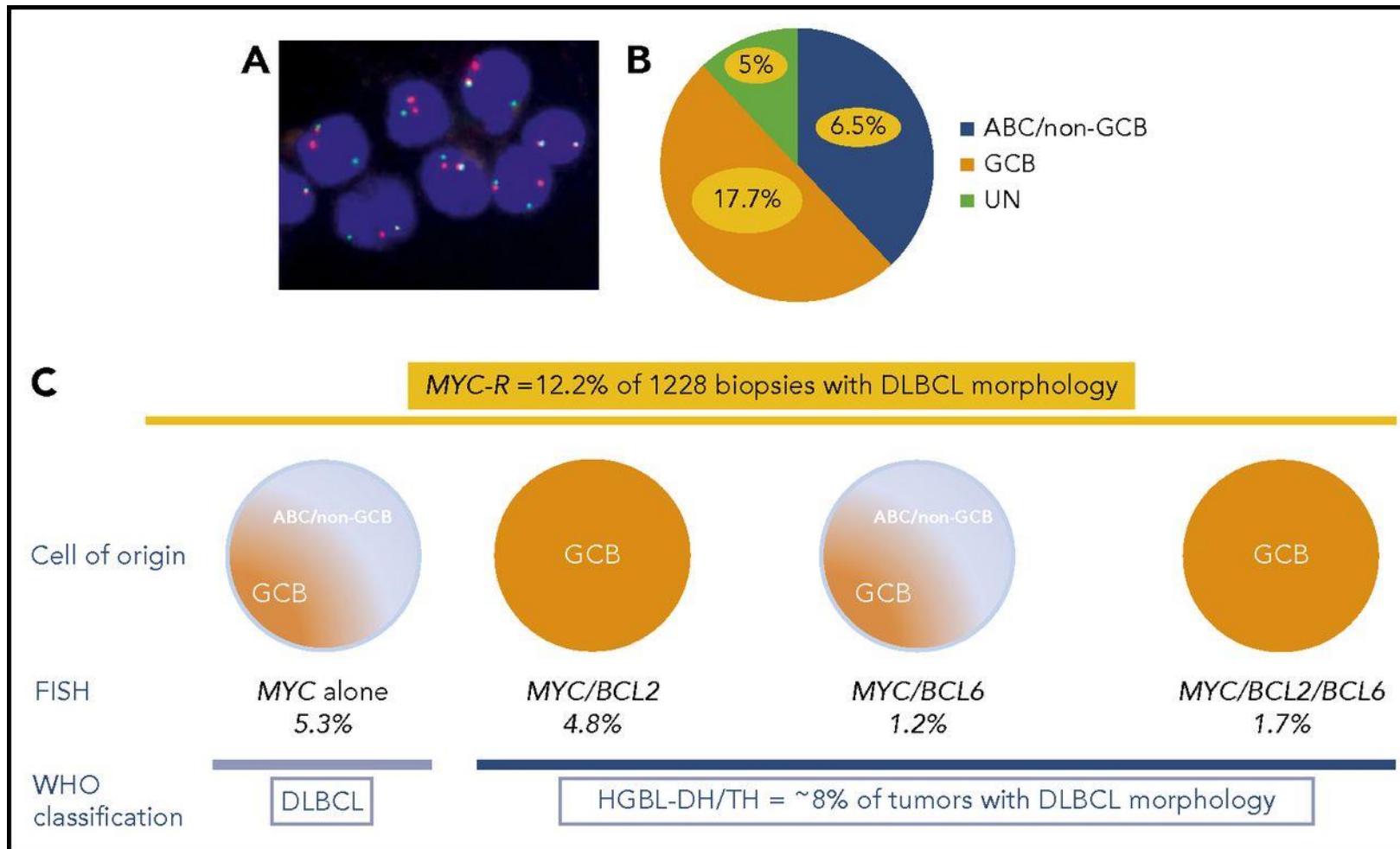
Somatic Mutations in PBL

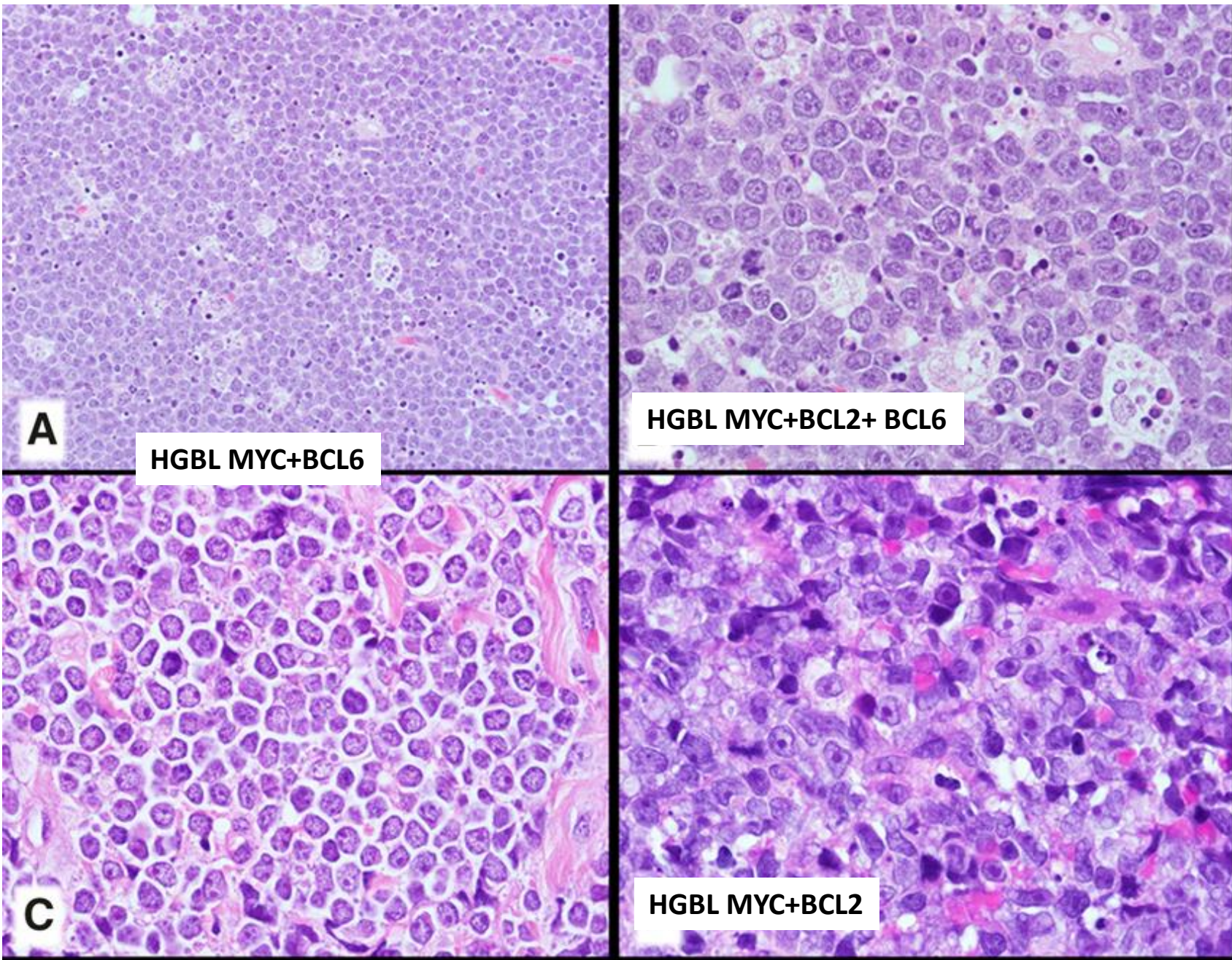
EBV+:

- More JAK/ STAT3 alterations
- Less TP53 mutations
- Less complex Karyotypes
- Worse outcome

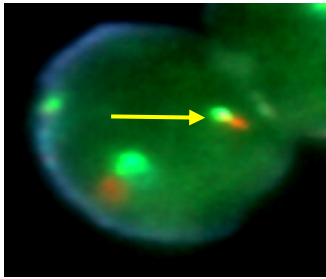


MYC Rearrangements in DLBCL

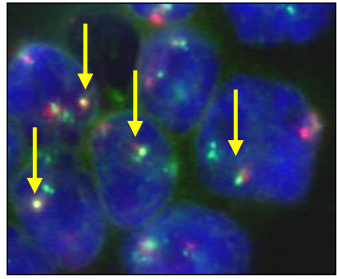




MYC / BCL2 Genetic vs Protein Double Expressors

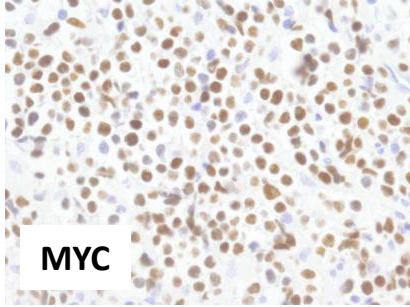


t(8;14)

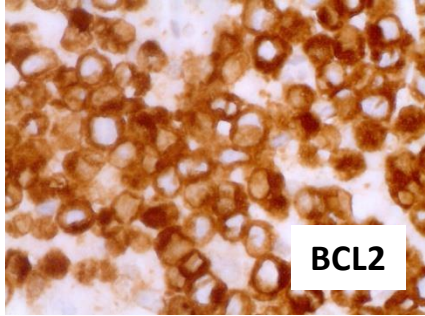


t(14;18)

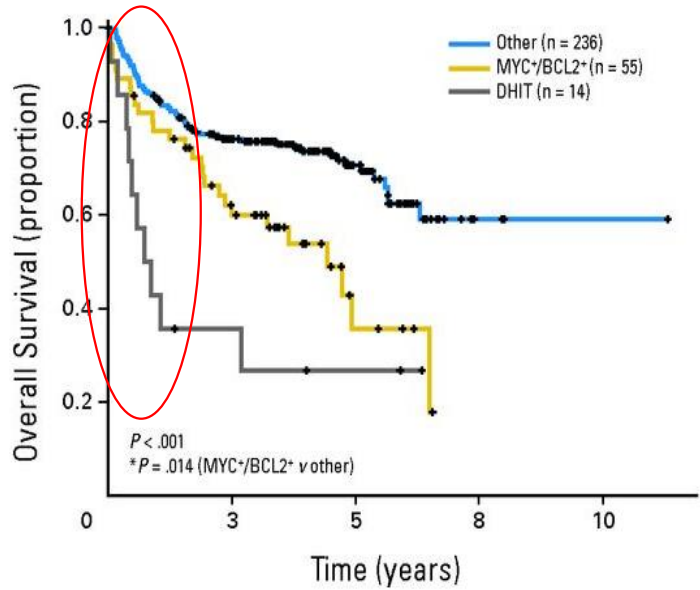
≠



MYC



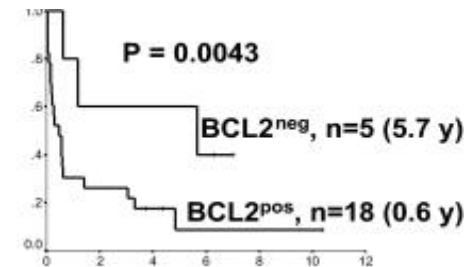
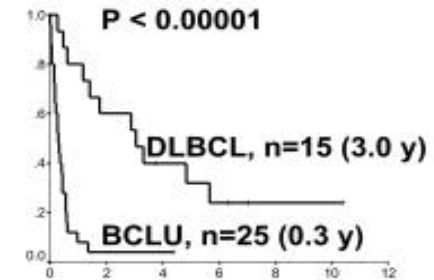
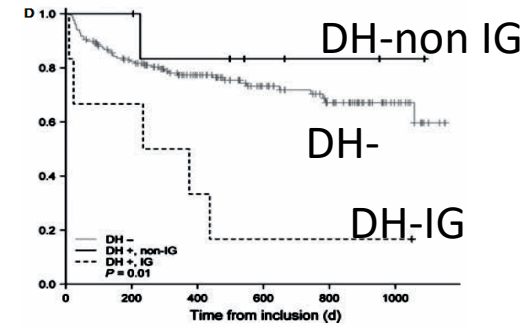
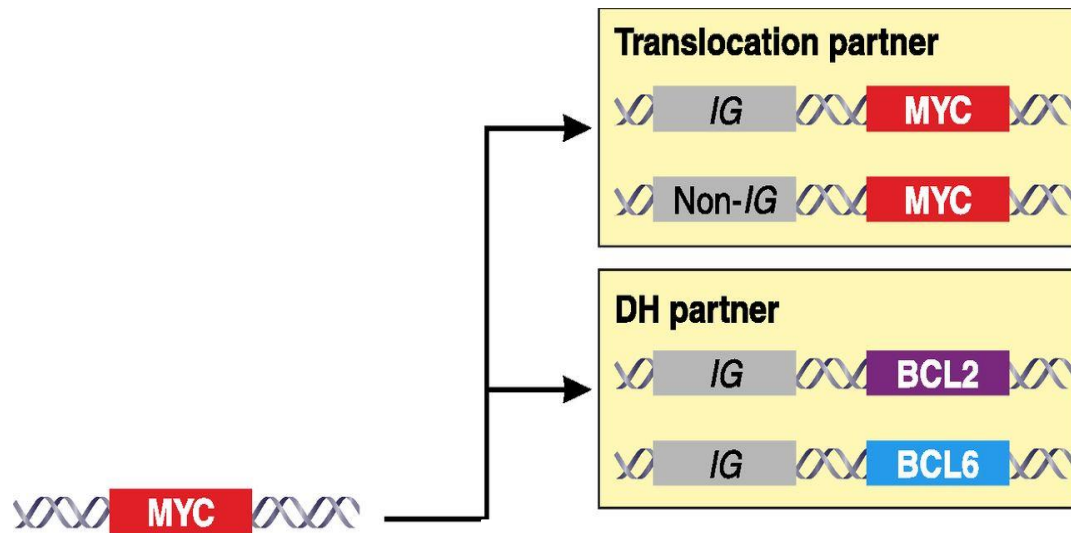
BCL2



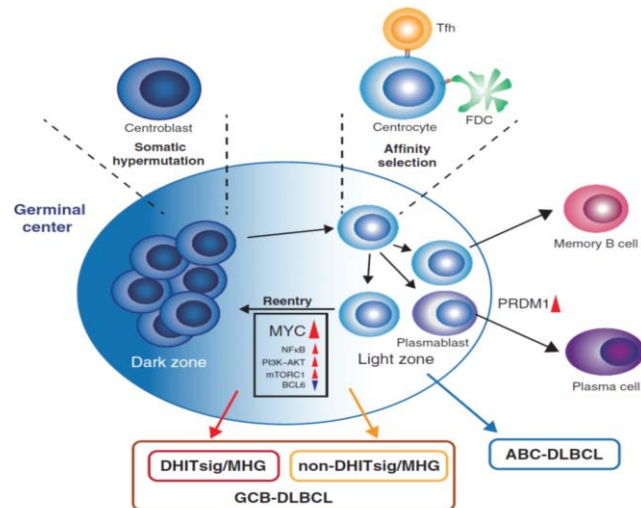
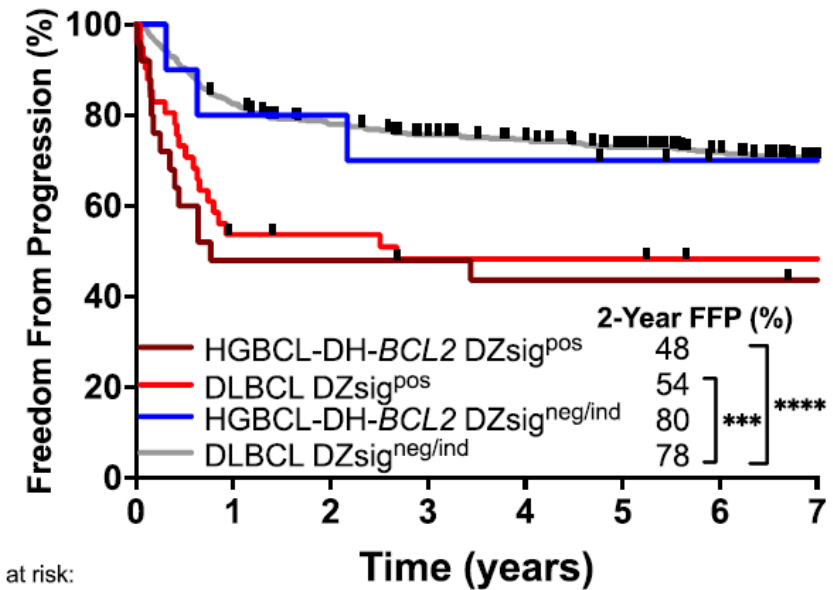
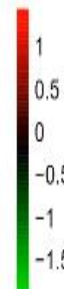
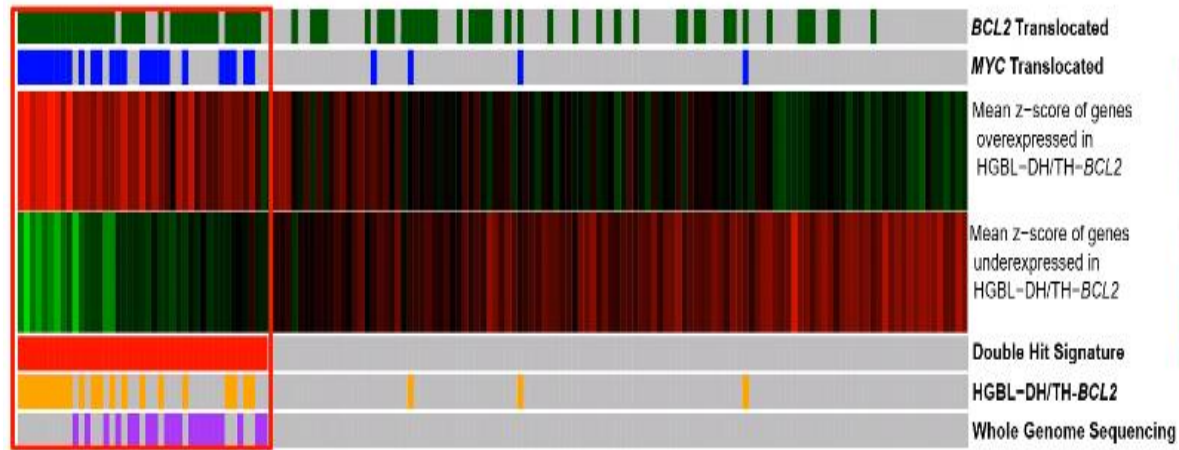
Johnson NA et al J Clin Oncol 2012

Not all “Double Hit” lymphomas are created equal

Modulators of the prognostic impact



Dark-zone gene expression signature identifies a subset of high grade lymphomas with and without BCL2-MYC rearrangements

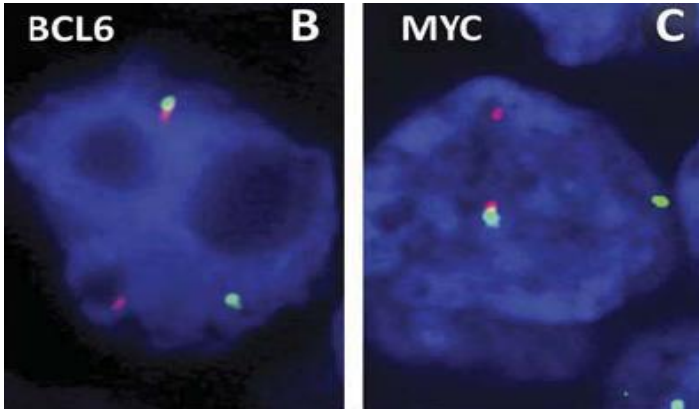


Ennishi D et al J Clin Oncol 2018; Sha Ch et al J Clin Oncol 2018; Hilton LK et al Blood 2019; Ennishi D et al Cancer Discov 2020; Alduaij W et al Blood 2022

Proposal for High grade B-cell Lymphomas

- **High-grade B-cell lymphomas with *MYC* and *BCL2* rearrangements (ICC and WHO HAEM-5)**
 - Specify whether DLBCL, blastoid or intermediate morphology
 - FISH break apart probes recommended but may miss up to 20% cases (cryptic)
 - IG or non-IG translocated partner inconclusive results
 - Do not consider CNA
 - Germinal center origin
 - Gene expression signature of centroblast in the GC dark zone
 - Mutational profile similar to “aggressive” FL (*BCL2*, ***MYC***, *KMT2D*, *CREBPP*, *TNFRS14*, *EZH2*)
- ***High-grade B-cell lymphoma with MYC and BCL6 rearrangements (ICC not in WHO HAEM-5)***
 - Heterogeneous in cell of origin and mutational profile (less FL –type, *NOTCH2*)
 - 30% may be “pseudo double” hit
- **High-grade B-cell lymphoma, NOS** (Blastoid or Intermediate cytology, no double rearrangements)

High Grade B-cell Lymphomas *MYC* & *BCL6* Rearrangements

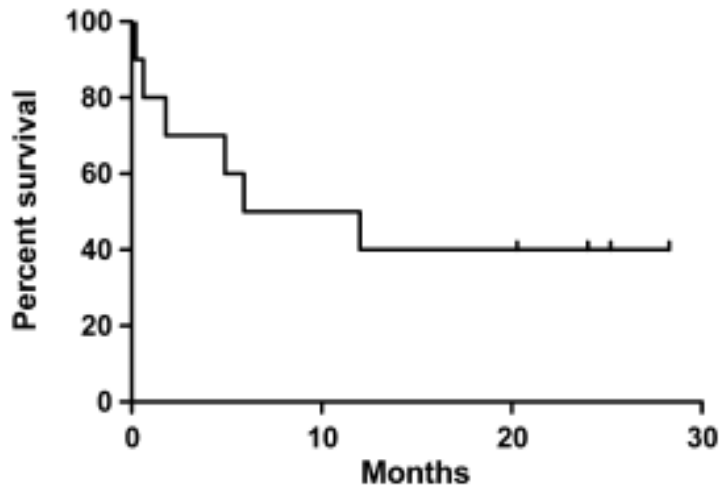


Compared to DLBCL (60 DH-BCL6 vs 217 DLBCL)

- Extranodal presentation
- Higher stage and IPI
- Worse outcome

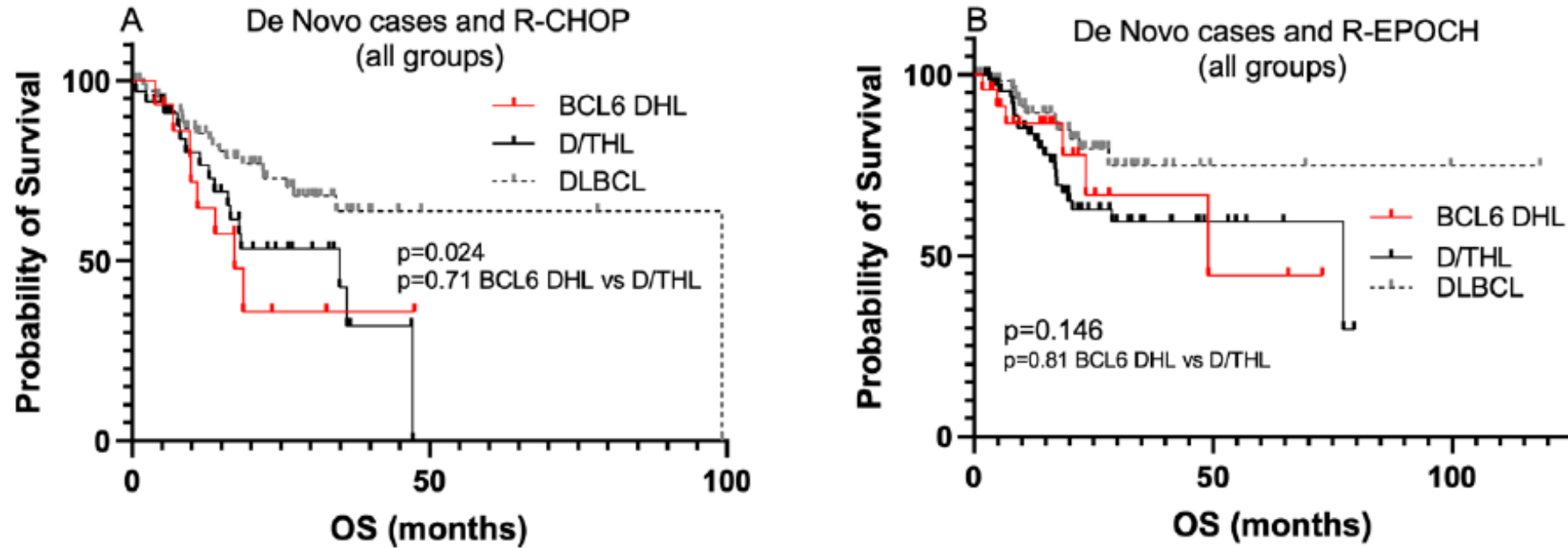
Compared to DH *MYC*-*BCL2*

- Intermediate DLBCL/BL morphology
- GCB and ABC phenotype
- Strong expression of BCL6
- Less frequent double BCL2 /MYC expression
- IRF4 +
- Similar por outcome



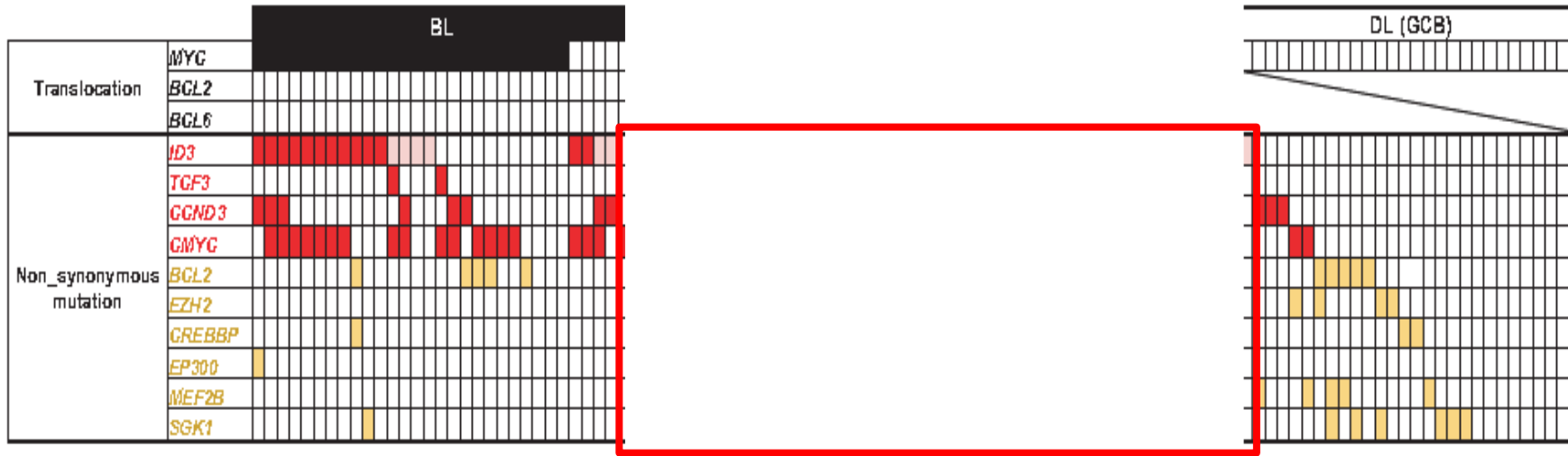
High Grade B-cell Lymphomas MYC & BCL6 (MD Anderson Cancer Ctr Houston Tx)

Figure 1: Overall survival of *BCL6*-DHL patients vs *BCL2*-DHL and DLBCL-NOS patients.



Conclusions: *BCL6*-DHL patients show aggressive clinical characteristics similar to *BCL2*-DHL patients and more aggressive than DLBCL patients. The major difference between *BCL6*-DHL and *BCL2*-DHL was immunophenotype, with *BCL6*-DHL having less often MYC and BCL2 double expression and GCB type. R-EPOCH, but not R-CHOP, improved the survival of *BCL6*-DHL patients, similar to *BCL2*-DHL patients. These data suggest that *BCL6*-DHL needs a separate recognition other than DLBCL for optimal patient management.

Burkitt and DLBCL Mutational profile in BCL-U Double-hit, and DLBCL with MYC translocations



- Mut BL: ***ID3, TCF3, CCND3, MYC***
- Mut DLBCL-GC: ***BCL2, EZH2, CREBB, MEF2B, SGK1***

Flow Chart for the Diagnosis of Aggressive B-cell Lymphomas

